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6 **Attenuation of melanogenesis by *Nymphaea nouchali***
7 **(Burm. f) flower extract through the regulation of**
8 **cAMP/CREB/MAPKs/MITF and proteasomal**
9 **degradation of tyrosinase**

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Table S1: List of the primer sets used in this study

Gene name		Sequences
<i>Tyrosinase</i>	<i>forward</i>	<i>TATGCGATGGAACACCTGAG</i>
	<i>reverse</i>	<i>ATAGGTGCATTGGCTTCTGG</i>
<i>TYRP1</i>	<i>forward</i>	<i>TCACTGATGCGGTCTTTGAC</i>
	<i>reverse</i>	<i>TTCGGGAGCTCCTCATAGTC</i>
<i>TYRP2</i>	<i>forward</i>	<i>CCCGACTGTAATCGGAAGAA</i>
	<i>reverse</i>	<i>TCCAGCCACAACAGATGGTA</i>
<i>MITF</i>	<i>forward</i>	<i>ACCCGTCTCTGGAAACTTGA</i>
	<i>reverse</i>	<i>TCCACAGAGGCCTTGAGAAT</i>
<i>Pmel 17</i>	<i>forward</i>	<i>GGCACACACACAATGGAAGT</i>
	<i>reverse</i>	<i>GCTTCTGCAGTTGGCATGTA</i>
<i>SLC24A5</i>	<i>forward</i>	<i>GGGCTCTATGTTCTGCTGCT</i>
	<i>reverse</i>	<i>TCTCCTGCAGTCTGGTGTG</i>
<i>SLC45A2</i>	<i>forward</i>	<i>CTAACCCAAGGCAGAAGCTG</i>
	<i>reverse</i>	<i>CGTATTCATGCATCCCCTG</i>
<i>OCA2</i>	<i>forward</i>	<i>GACATGCGCCTAGAGAACAA</i>
	<i>reverse</i>	<i>AGCAACCTCTTTACCCAGCA</i>
<i>VDAC</i>	<i>forward</i>	<i>TGGGAAATTAAGGCCTCCT</i>
	<i>reverse</i>	<i>GCTGTCCATGCCAGGTTTAT</i>
<i>TRPM1</i>	<i>forward</i>	<i>AGACCATGTCCAACCCTCTG</i>
	<i>reverse</i>	<i>CGCAGTATTTGTGTGCGAAG</i>
<i>Gapdh</i>	<i>Forward</i>	<i>TTGTGATGGGTGTGAACCAC</i>
	<i>reverse</i>	<i>ACACATTGGGGGTAGGAACA</i>

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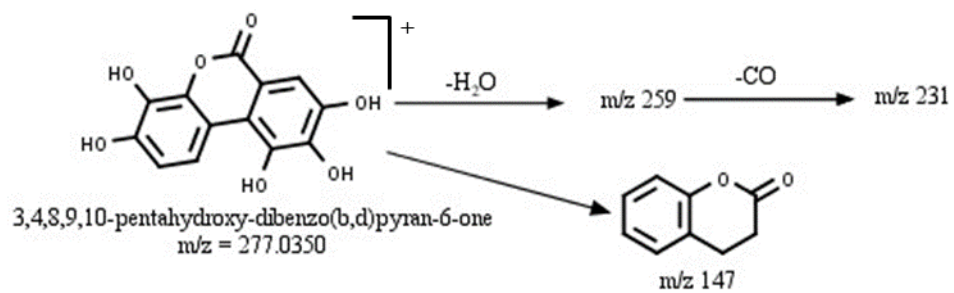
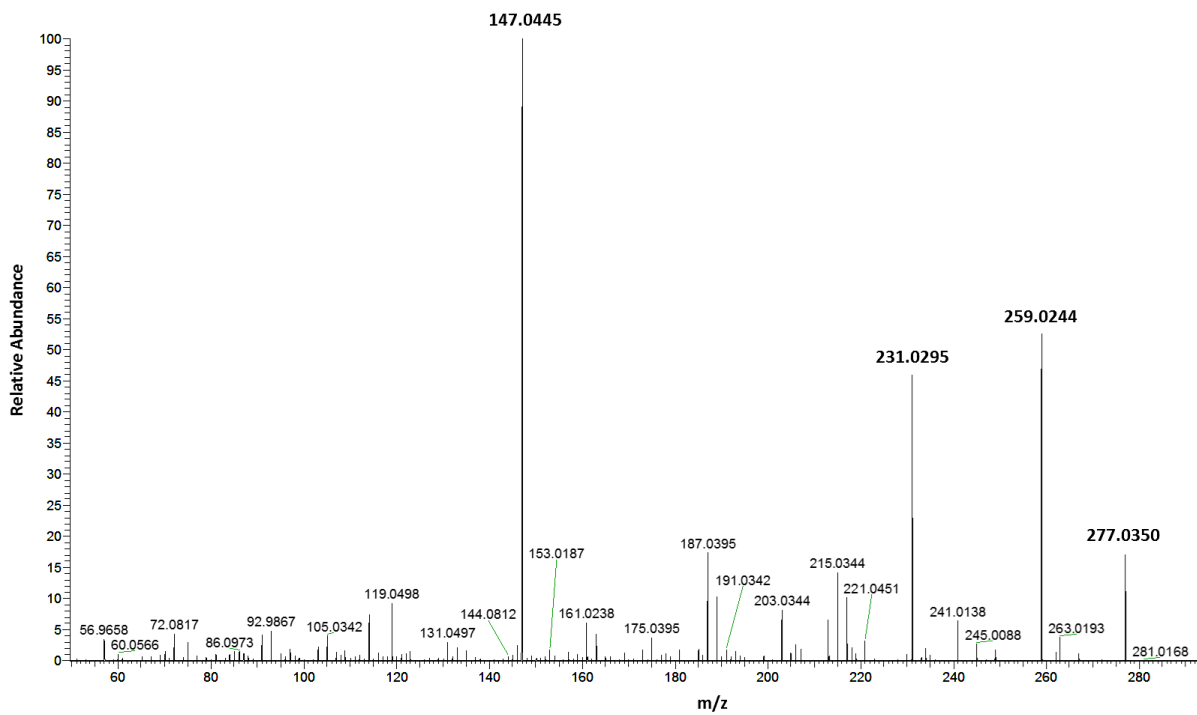


Figure S1: Chemical Structure of 3,4,8,9,10-pentahydroxy-dibenzo(b,d)pyran-6-one and possible mass fragmentation

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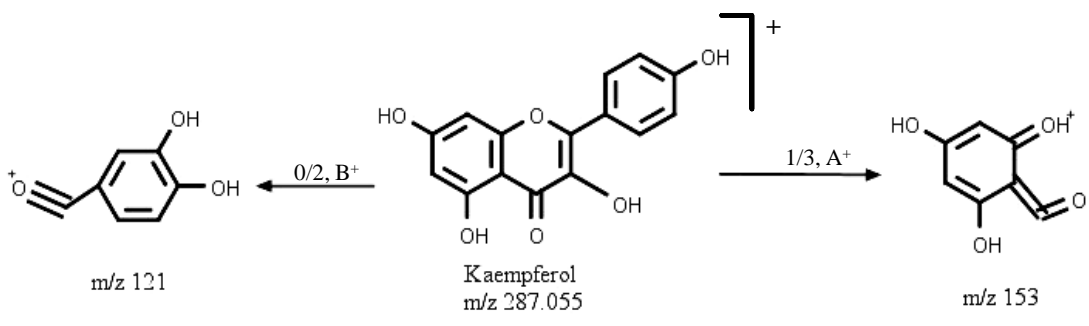
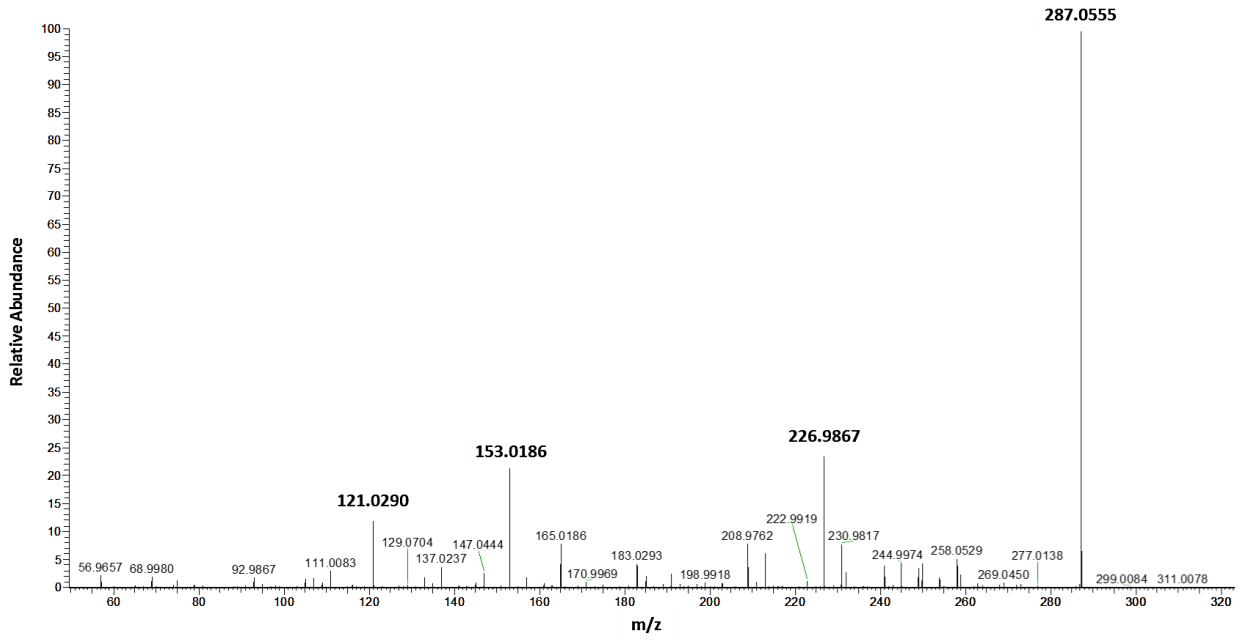


Figure S2: Chemical Structure of Kaempferol and the major possible mass fragmentation

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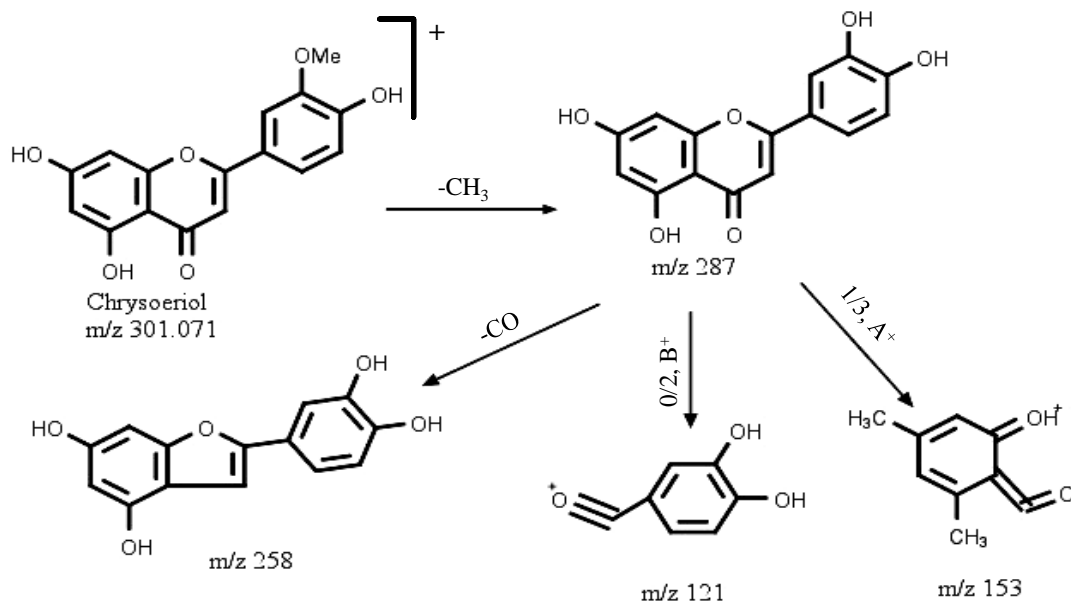
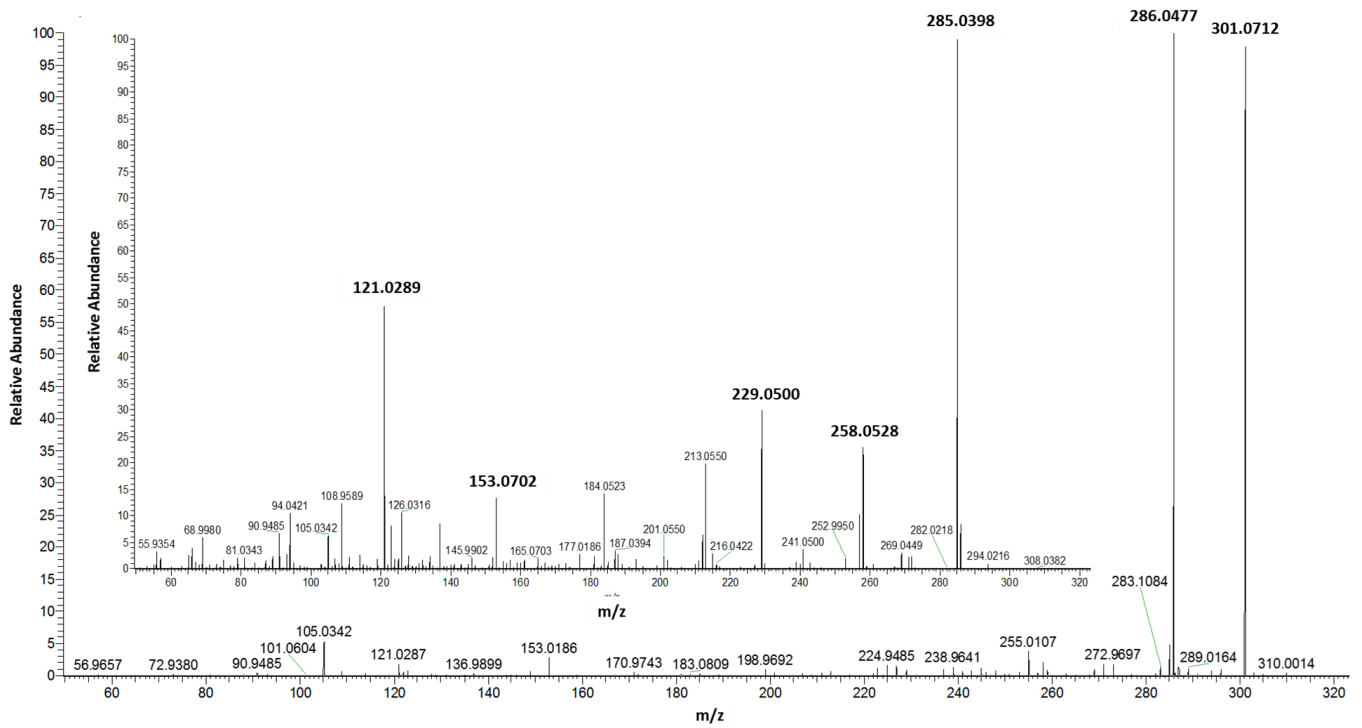


Figure S3: Chemical Structure of chrysoeriol and the major possible mass fragmentation

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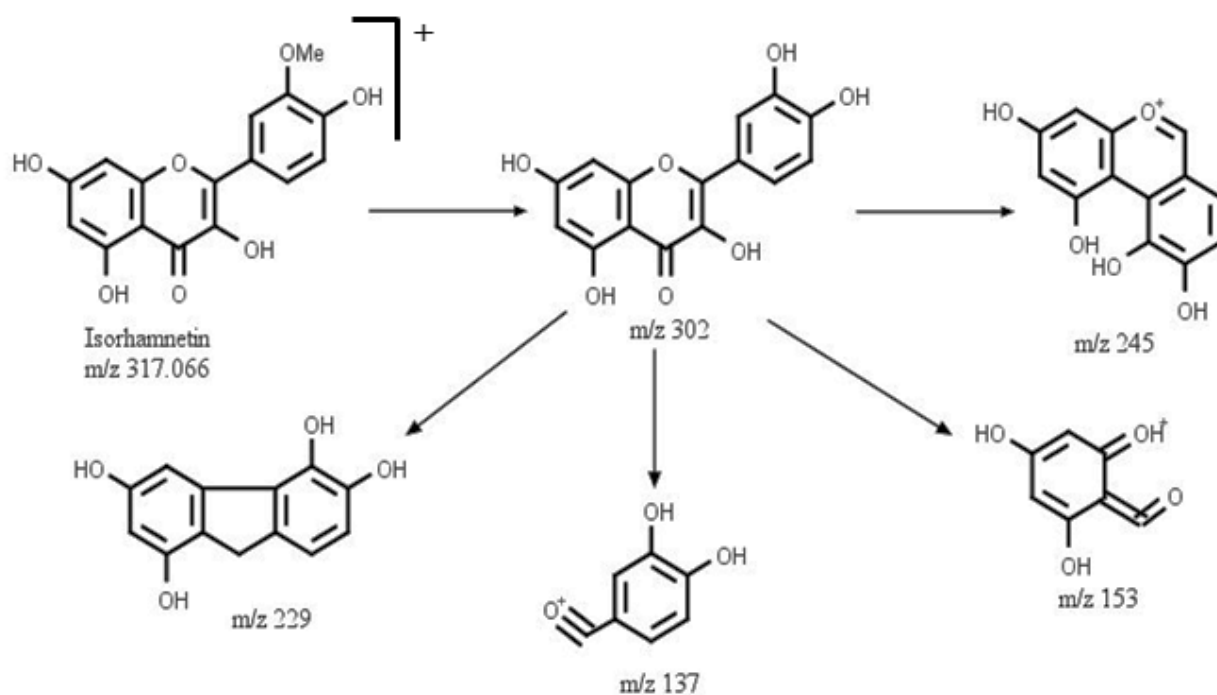
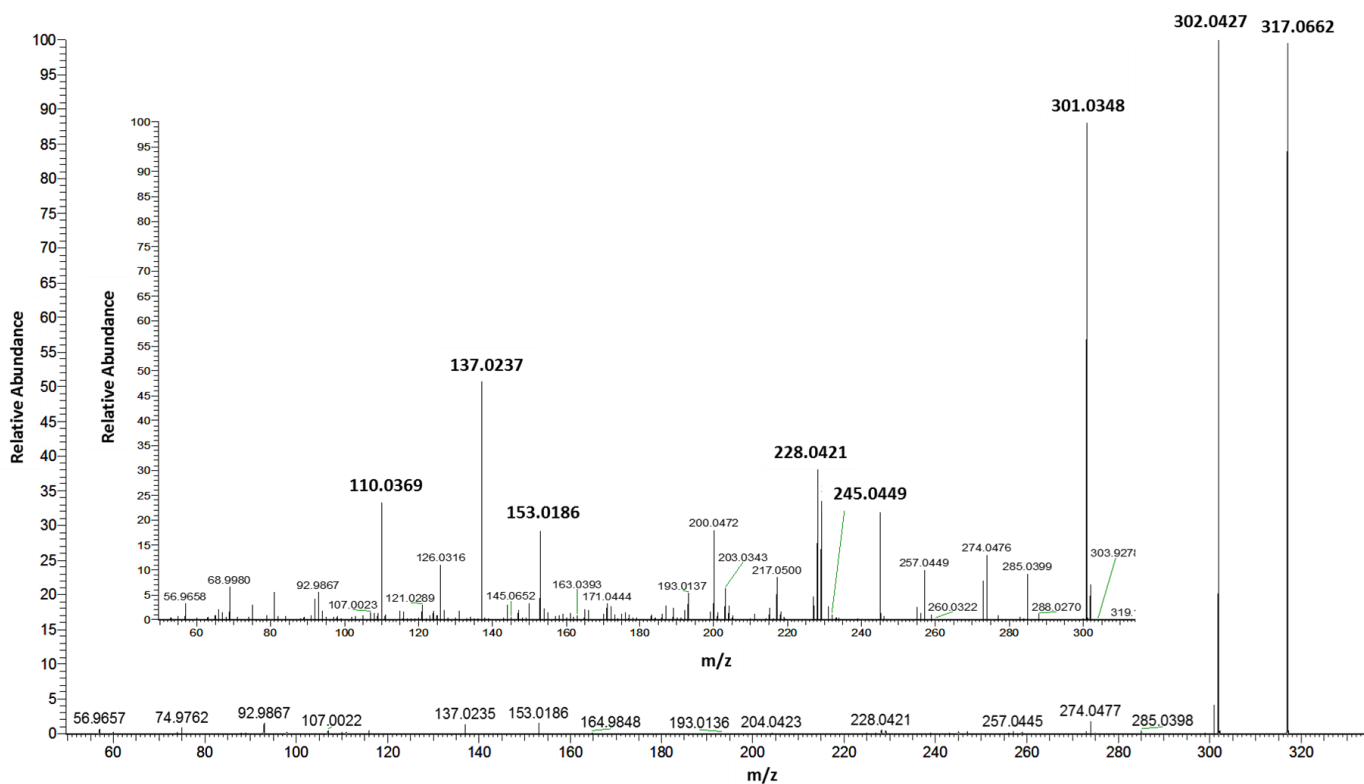
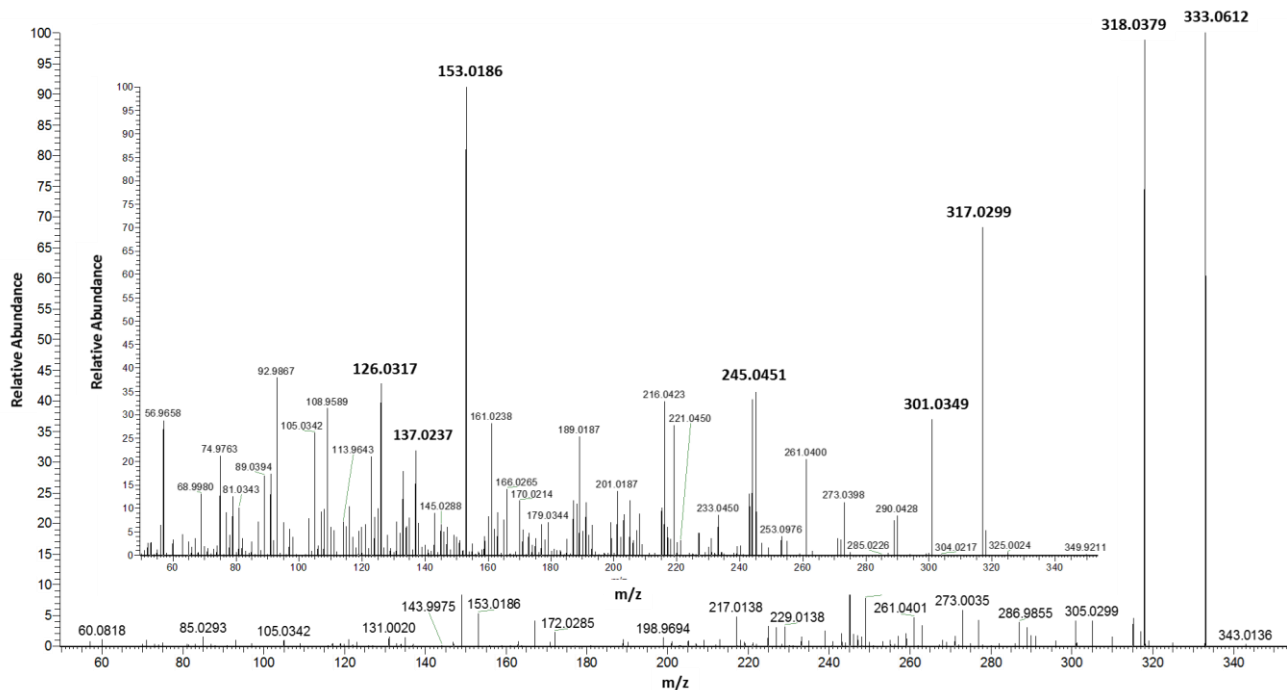


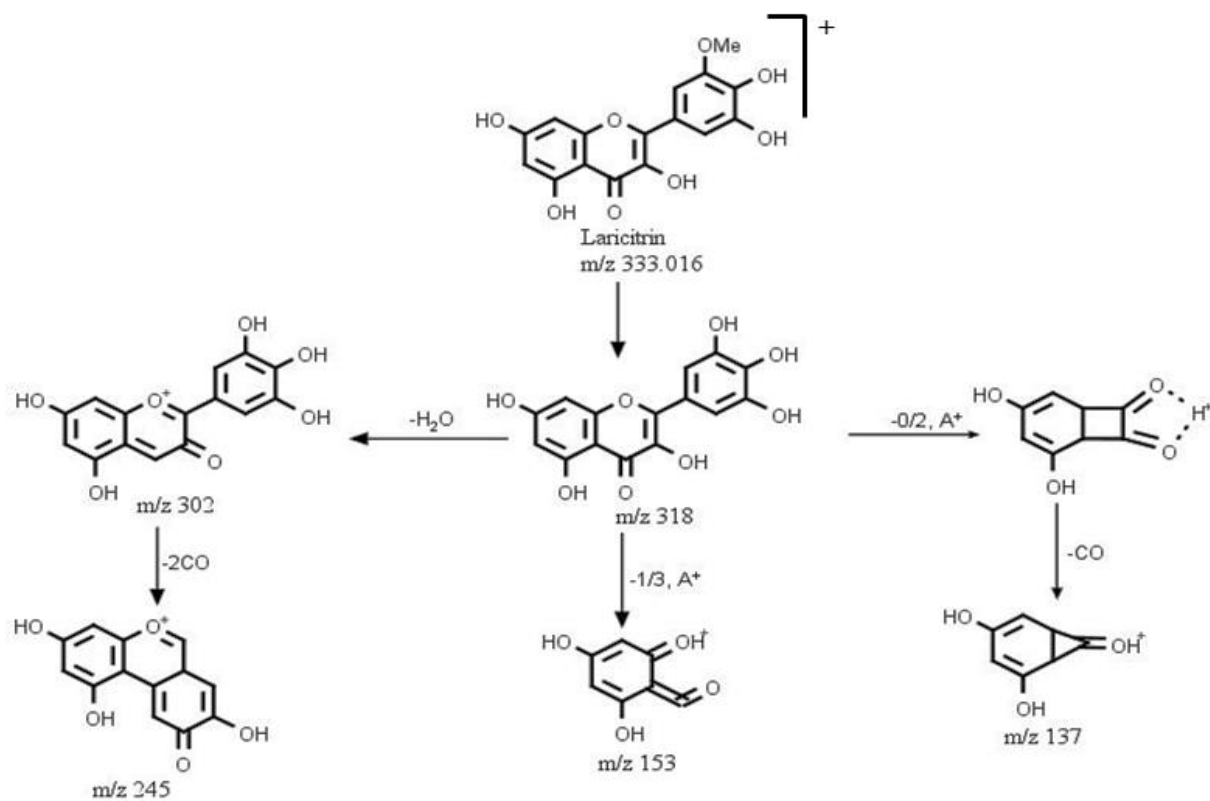
Figure S4: Chemical Structure of isorhamnetin and the major possible mass fragmentation

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156 **Figure S5:** Chemical Structure of laricitrin and the major possible mass fragmentation

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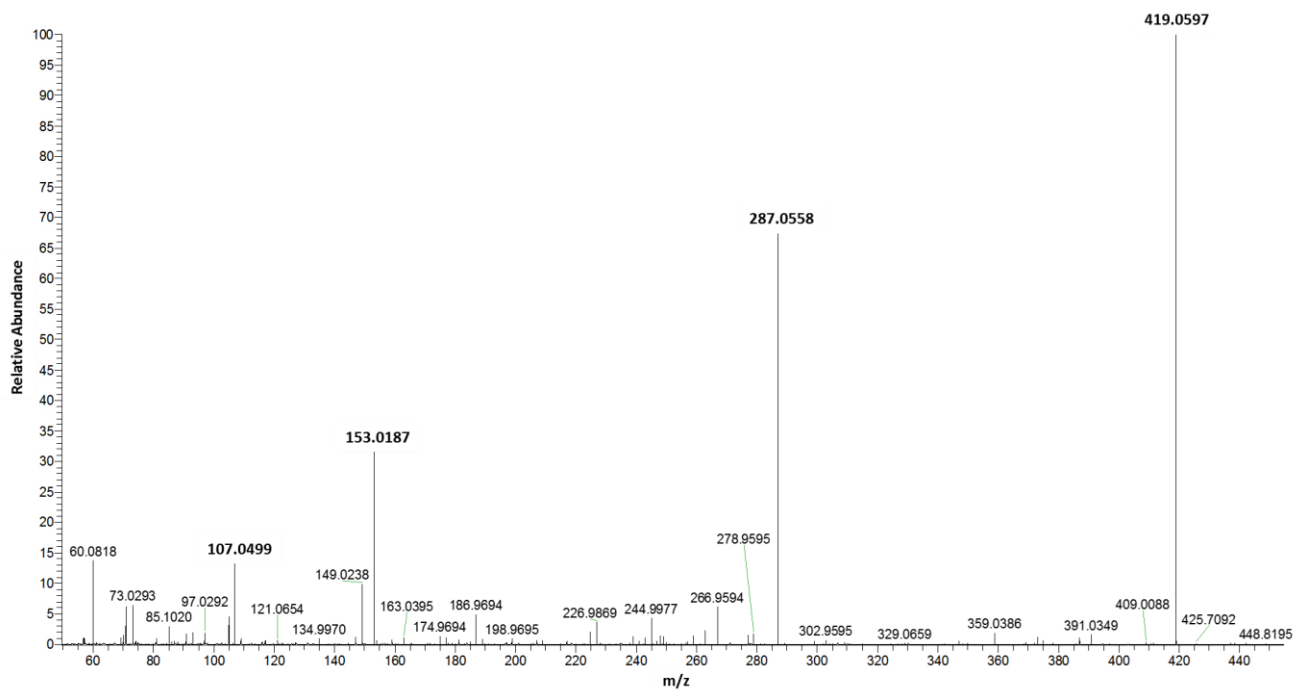
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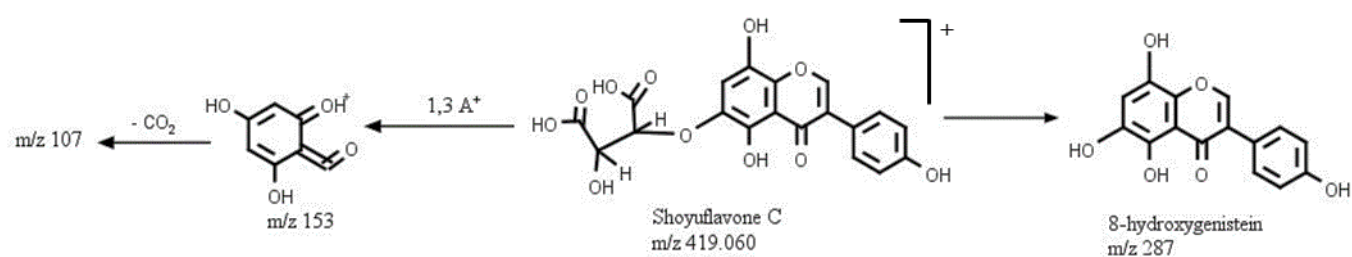


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174 **Figure S6:** Chemical Structure of shoyuflavone C and the major possible mass fragmentation

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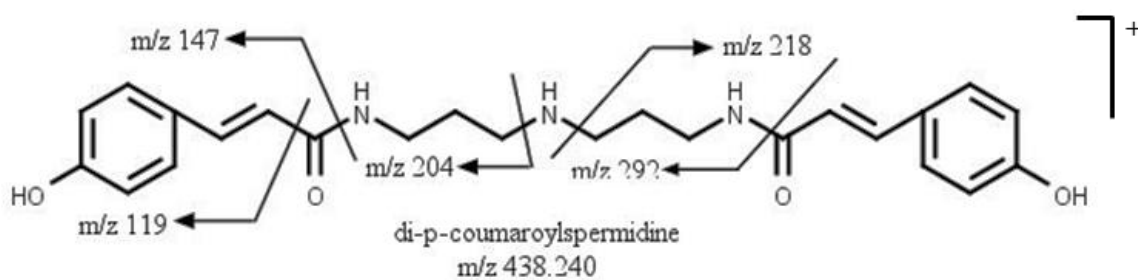
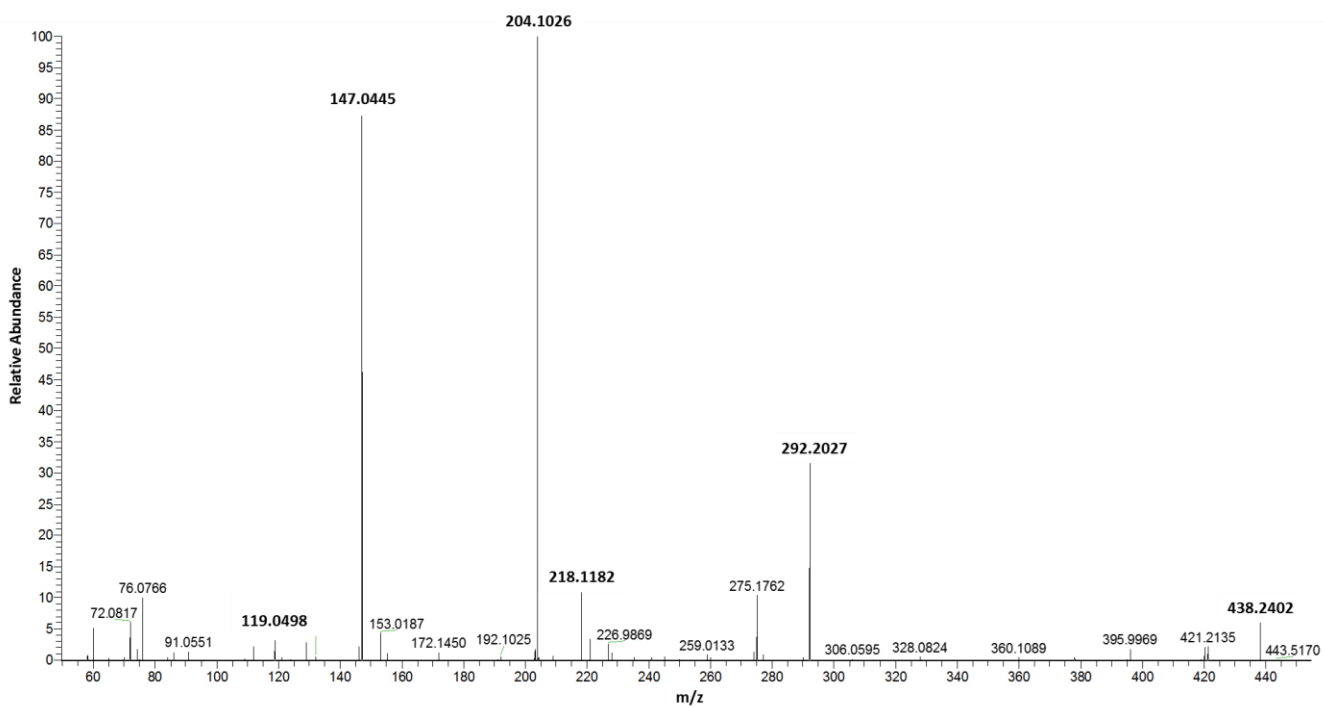
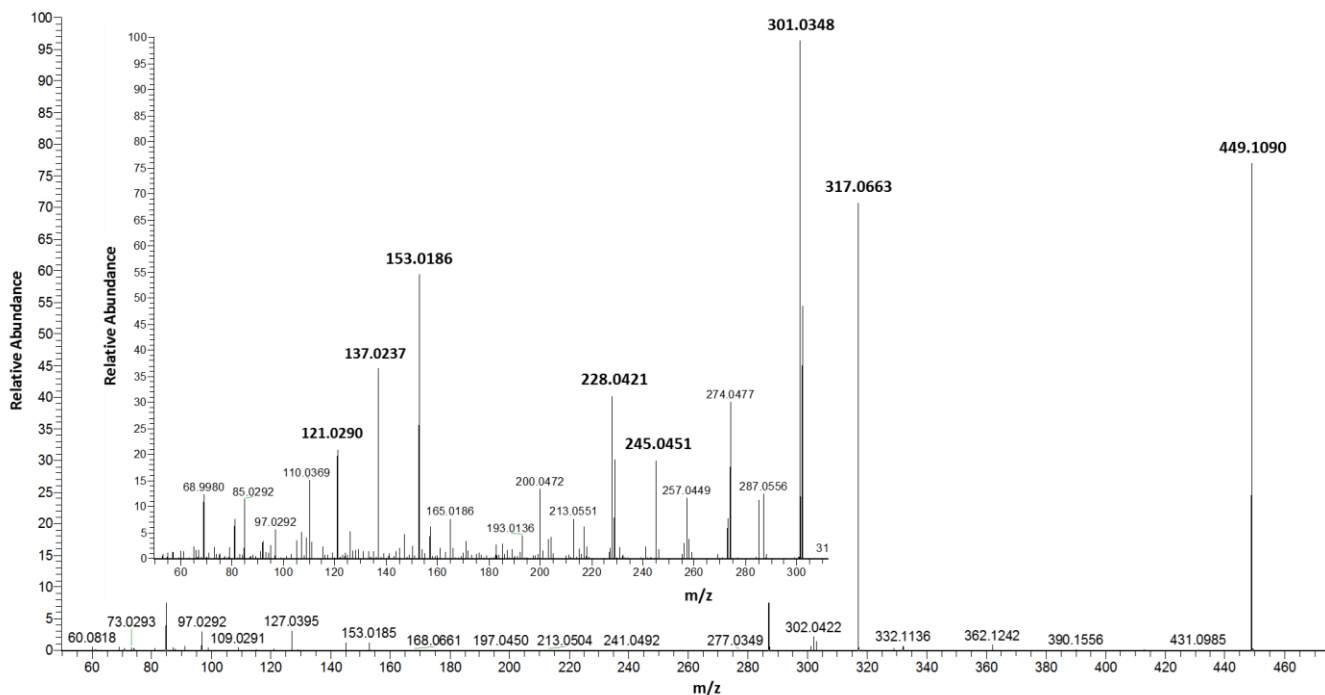


Figure S7: Chemical Structure of di-*p*-coumaroylspermidine and the major possible mass fragmentation

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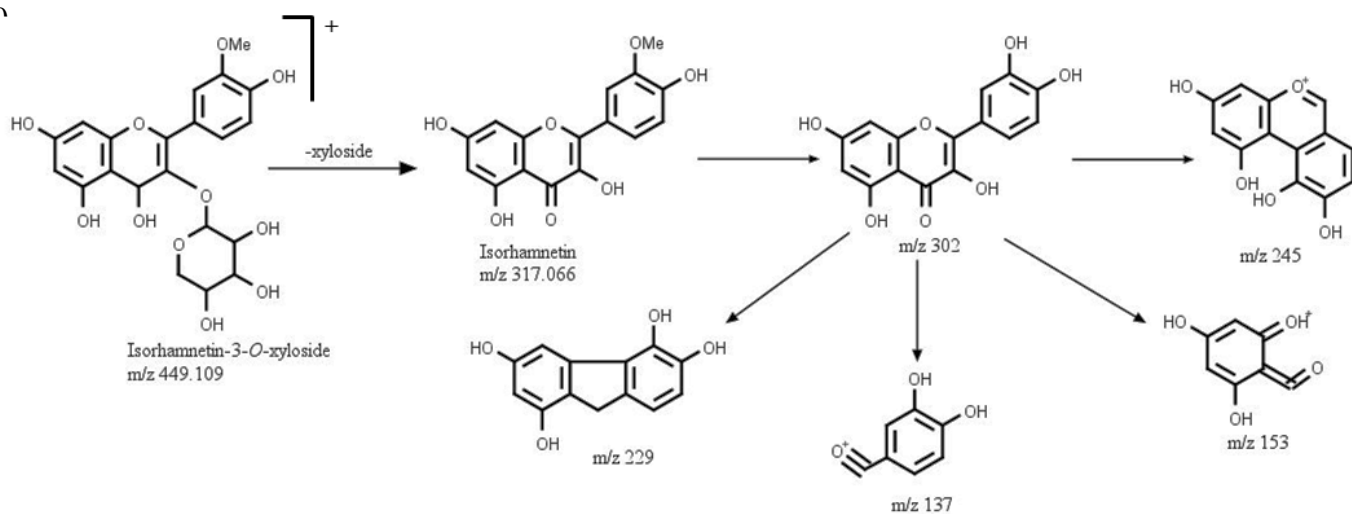


Figure S8: Chemical Structure of isorhamnetin-3-O-xyloside and the major possible mass fragmentation

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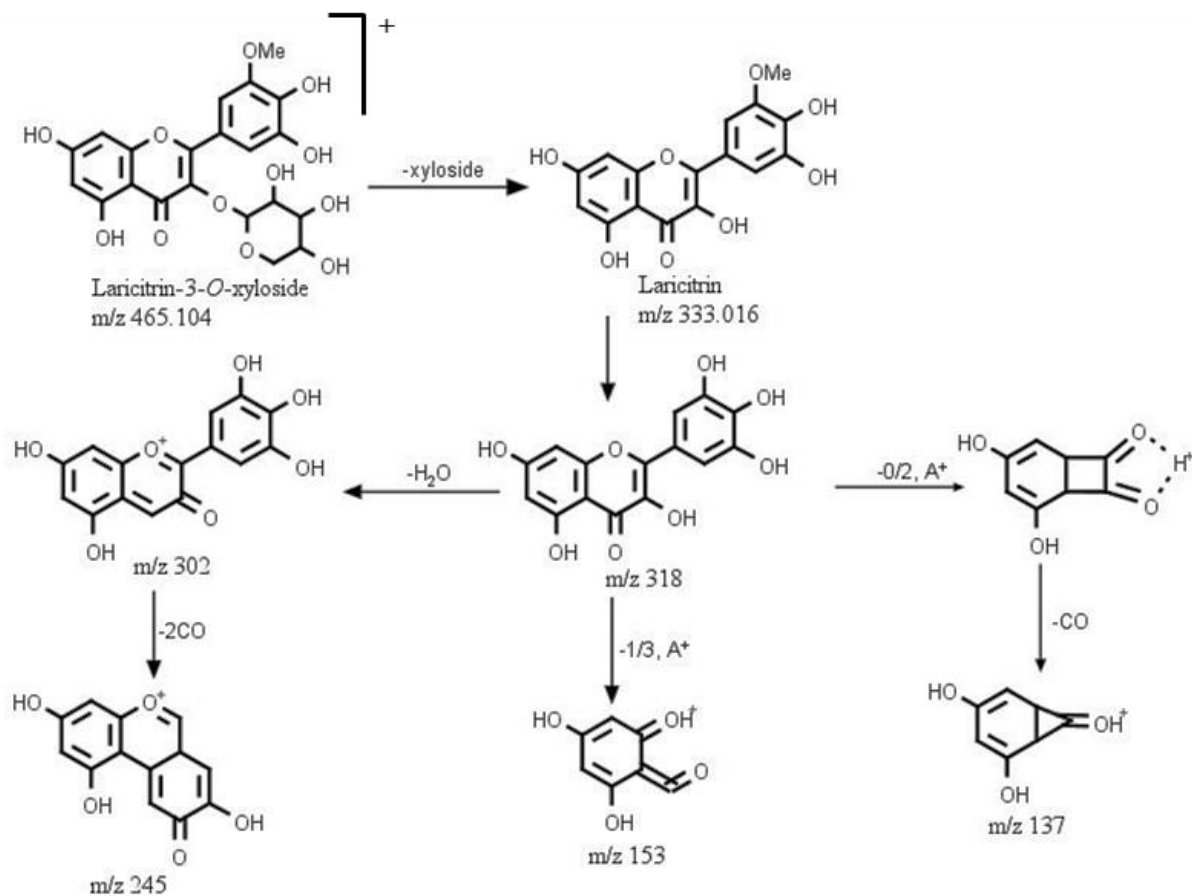
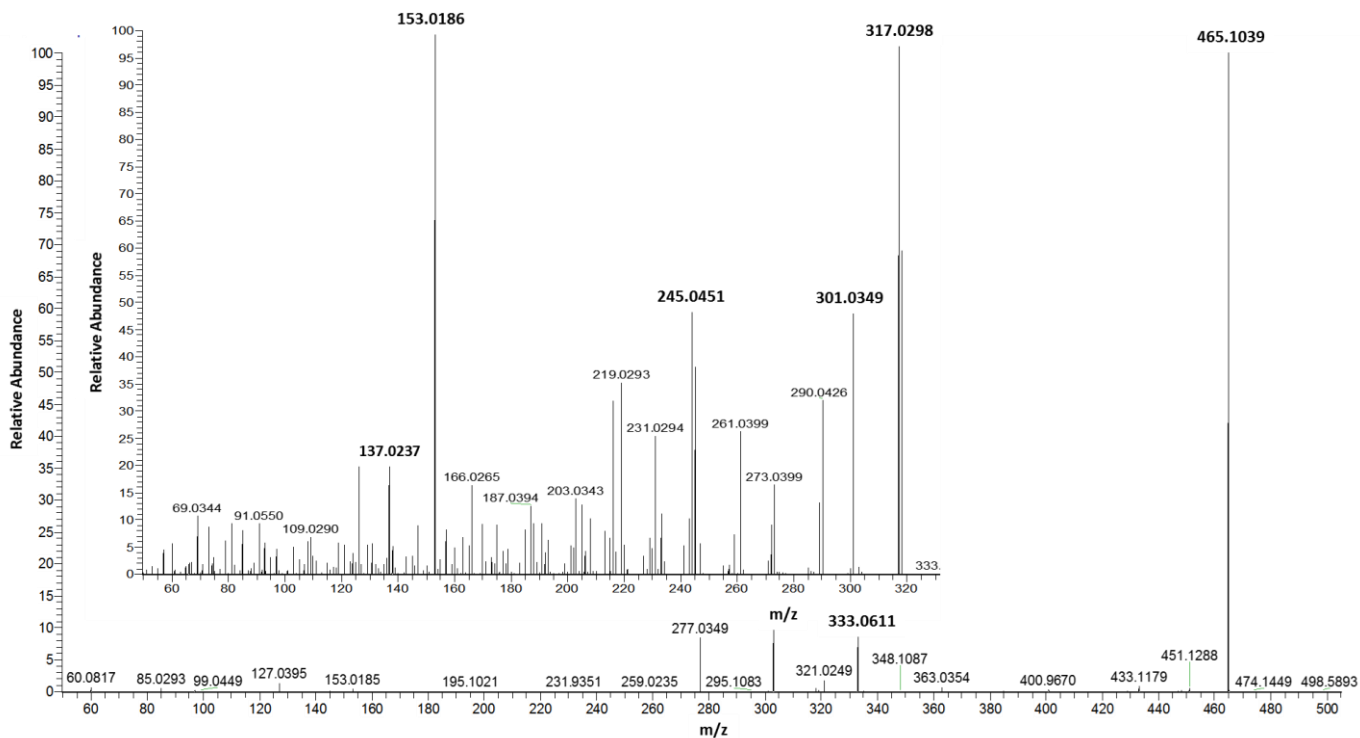


Figure S9: Chemical Structure of laricitrin-3-O-xyloside and the major possible mass fragmentation

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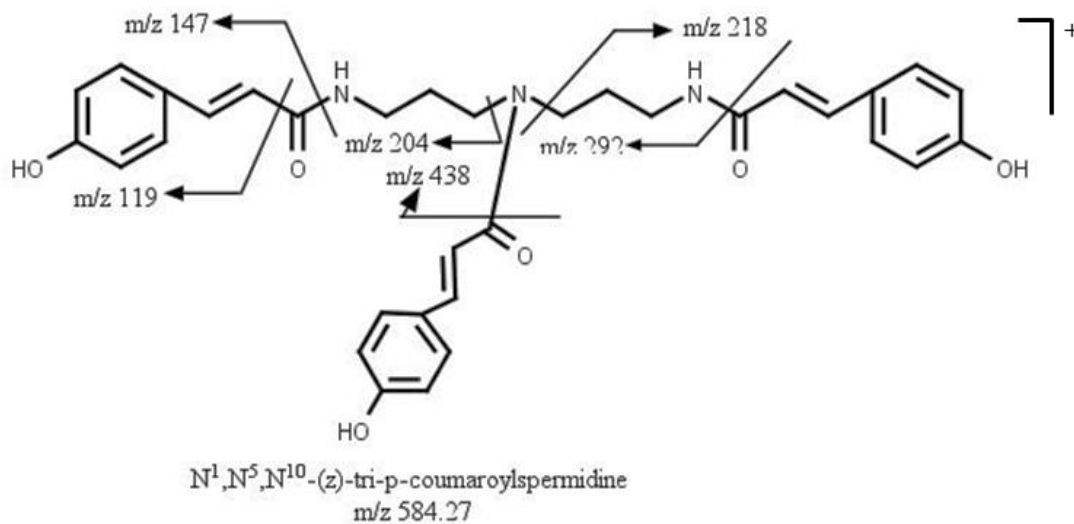
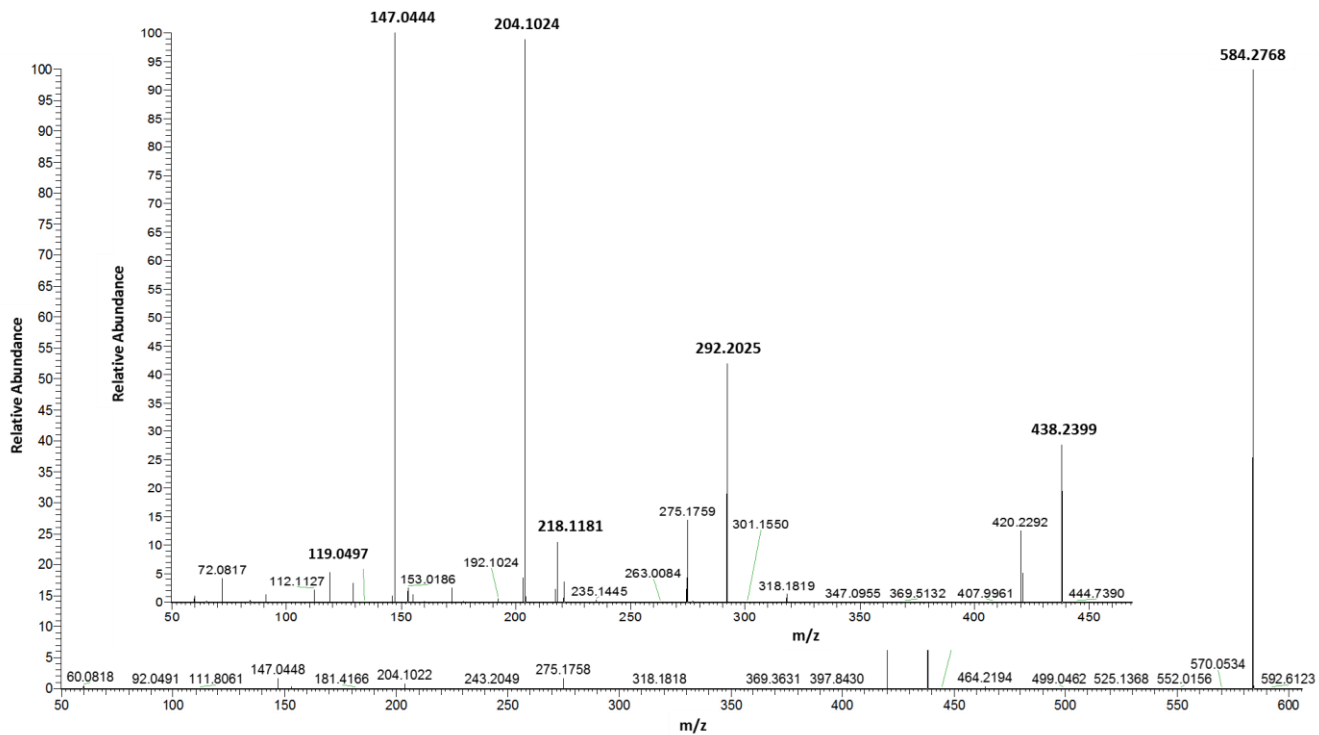
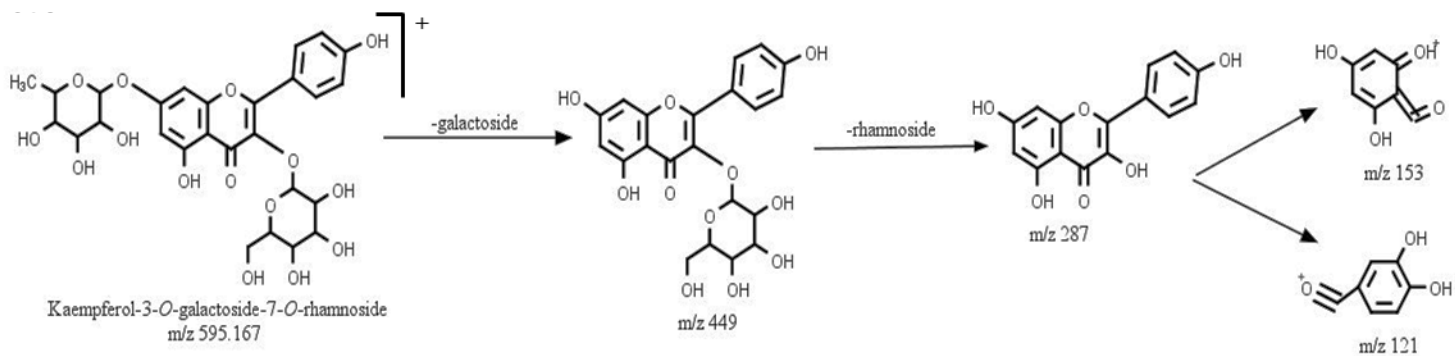
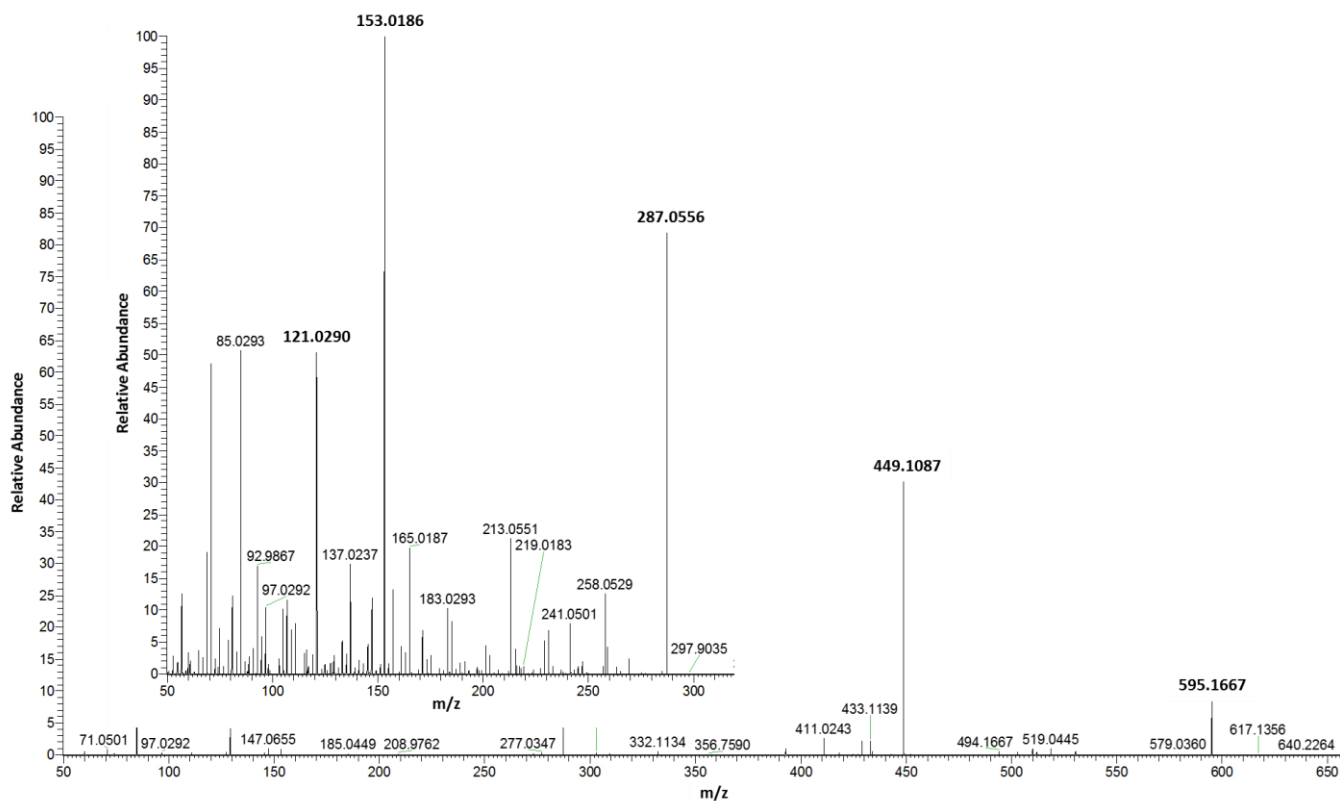


Figure S10: Chemical Structure of N^1, N^5, N^{10} -(z)-tri-p-coumaroylspermidine and the major possible mass fragmentation

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299 **Figure S11:** Chemical Structure of kaempferol-3-O-galactoside-7-O-rhamnoside and the
300 major possible mass fragmentation

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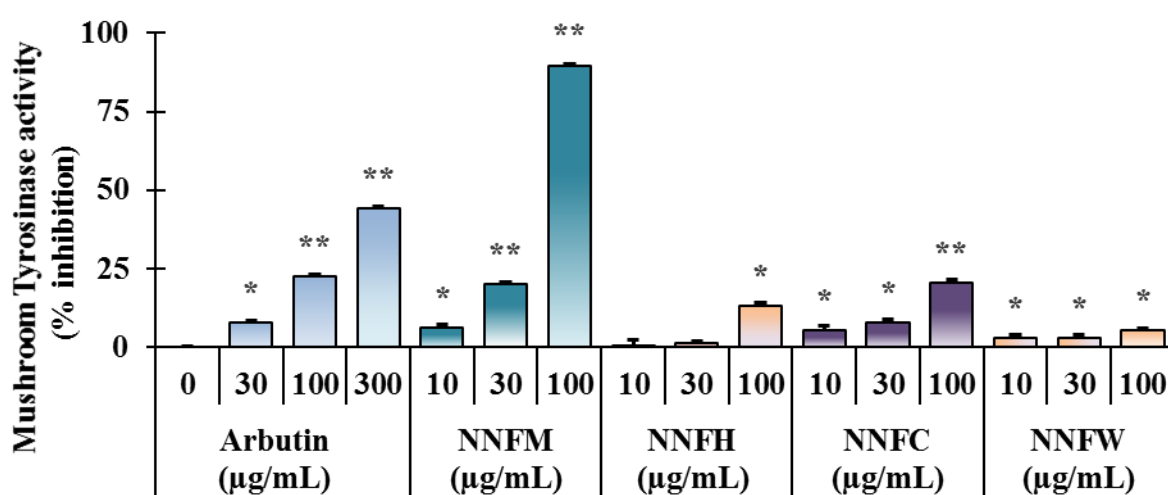


Figure S12: Mushroom tyrosinase inhibition activities of methanolic extract and various organic solution fractions of *Nymphaea nouchali* flowers. Values are expressed as the mean \pm SD (n=3). *p < 0.05 and **p < 0.01, significantly different from control, using student's t-test.

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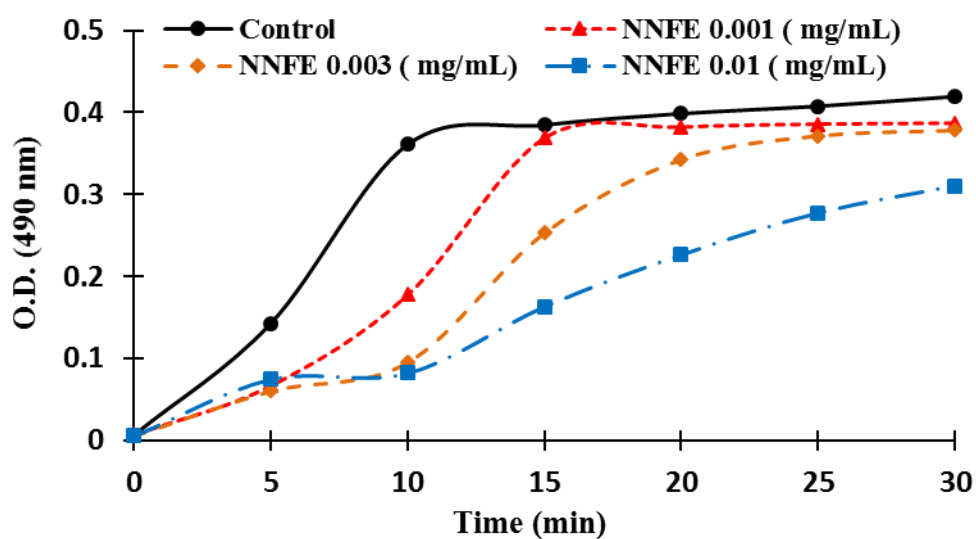


Figure S13: Mushroom tyrosinase inhibition kinetics of ethyl acetate fractions of *Nymphaea nouchali* flowers (NNFE) in which substrate is L-DOPA. Values are expressed as the mean \pm SD (n=3).

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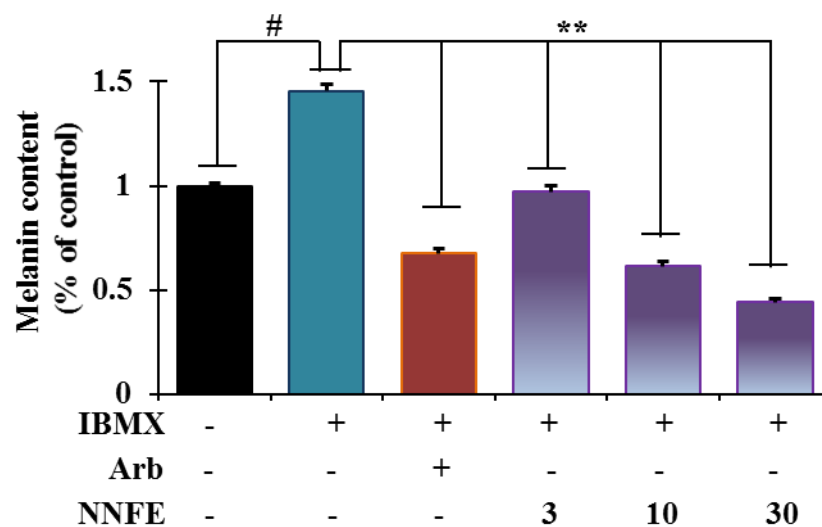


Figure S14: Cells were cultured with NNFE (3-30 $\mu\text{g ml}^{-1}$) for 3 days. IBMX induced melanin content were measured. Values are expressed as the mean \pm SD (n=3). #p < 0.05 significantly different from control and **p < 0.01, significantly different from IBMX-induced control, using student's t-test.

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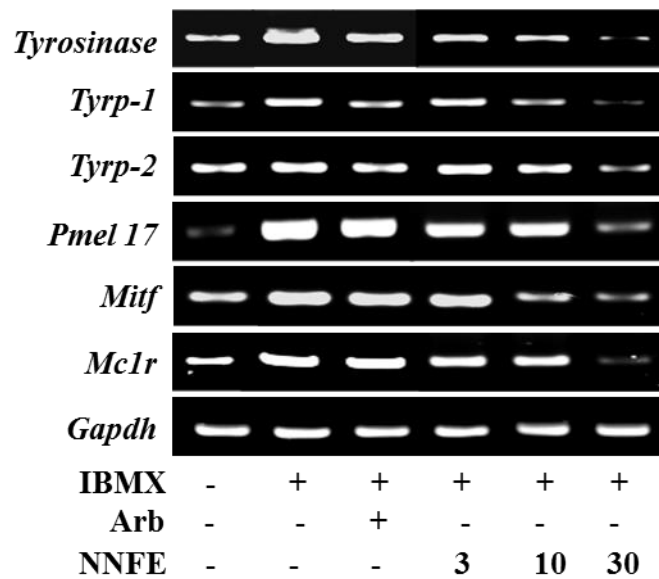
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398 Figure S15: Cells (5×10^5 cells ml^{-1}) were cultured for 24 h, and the medium was then

399 replaced with fresh medium containing the indicated concentrations of NNFE or arbutin for

400 24 h. mRNA was extracted using TRIzol and mRNA expressions was analyzed by RT PCR.

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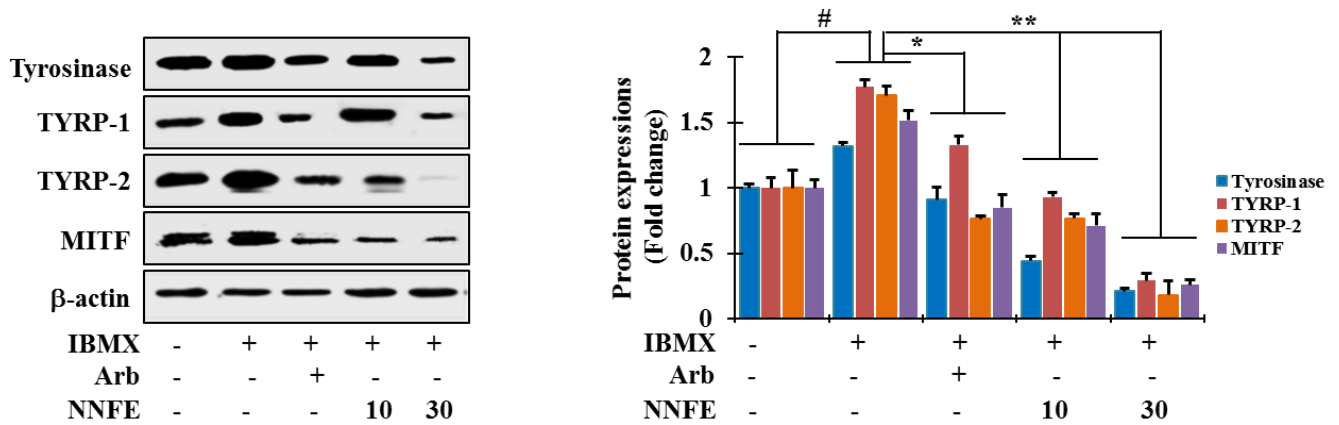


Figure S16: Cells (1×10^5 cells ml^{-1}) were cultured for 24 h; medium was replaced with fresh medium containing the indicated concentrations of NNFE or arbutin for 3 days. Total cell lysates were extracted and assayed by western blotting using antibodies against tyrosinase, TYRP-1, TYRP-2, and MITF. Equal protein loadings were confirmed using β -actin. Arb: Arbutin. Statistical analysis of the band intensity of tyrosinase, TYRP-1, TYRP-2, and MITF obtained by western blot analysis. #p < 0.05 significantly different from control and **p < 0.01, significantly different from IBMX-induced control, using student's t-test.

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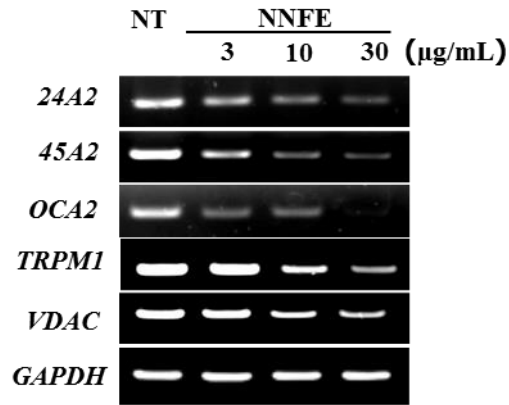
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440 Figure S17: Cells (5×10^5 cells ml^{-1}) were cultured for 24 h, and the medium was then

441 replaced with fresh medium containing the indicated concentrations of NNFE for 24 h.

442 mRNA was extracted using TRIzol and mRNA expressions was analyzed by RT PCR.

443