SUPPORTING INFORMATION:

# The Effect of *Aspalathus linearis* (Burm.f.) R.Dahlgren and its Compounds on Tyrosinase and Melanogenesis

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# S1 - Quantification of Pure Compounds Using Ultra-Performance Liquid Chromatography – Quantitative Time of Flight

The spectral data and spectra regarding the Ultra-Performance Liquid Chromatography – Quantitative Time of Flight analysis is provided in Table S 1, and Figure S 1 and S 2.

**Table S 1.** Spectral data of the major secondary chemical compounds present in the ethanolic extract of unfermented *Aspalathus linearis* (Burm.f.) R.Dahlgren and its fractions quantified using Ultra-Performance Liquid Chromatography – Quantitative Time of Flight

| Chemical compounds | Mode | Calibration curve | $R^2$ | LOD[a] (ppm) | LOQ[b] (ppm) |
|---|---|---|---|---|---|
| Aspalathin | Positive | y = 26.88x | 0.999 | < 0.25 | < 2.5 |
| Caffeic acid | Negative | y = 112.22x - 7.66 | 0.999 | < 0.25 | < 2.5 |
| Catechin | Negative | y = 15.02x - 10.97 | 0.999 | < 0.25 | < 2.5 |
| Cinnamic acid | Positive | y = 0.79x + 2.02 | 0.997 | < 0.25 | < 2.5 |
| (-)-Epicatechin | Negative | y = 26.56x - 19.68 | 0.999 | < 0.25 | < 0.25 |
| Ferulic acid | Negative | y = 31.39x + 0.75 | 0.994 | < 0.25 | < 2.5 |
| 4-hydroxy benzoic acid | Negative | y = 26.00x + 0.13 | 0.999 | < 25 | < 25 |
| Isoquercitrin | Negative | y = 95.22x - 50.05 | 0.999 | < 25 | < 25 |
| Luteolin | Positive | y = 0.0545x + 0.23 | 0.999 | < 0.25 | < 2.5 |
| Orientin | Positive | y = 0.03x + 0.26 | 0.981 | < 0.25 | < 2.5 |
| *p*-Coumaric acid | Negative | y = 106.42x - 3.15 | 0.993 | < 0.25 | < 2.5 |
| *n*-Propyl gallate | Negative | y = 336.83x - 56.28 | 0.992 | < 0.25 | < 0.25 |
| Quercetin | Positive | y = 93.33x - 90.54 | 0.995 | < 2.5 | < 2.5 |
| Rosmarinic acid | Negative | y = 63.19x - 8.43 | 0.995 | < 25 | < 25 |
| Syringic acid | Negative | y = 25.24x - 3.36 | 0.994 | < 0.25 | < 2.5 |
| Vanillic acid | Negative | y = 1.55x - 0.003 | 0.999 | < 0.25 | < 2.5 |
| Vitexin | Positive | y = 195.37x -175.73 | 0.996 | < 0.025 | < 0.25 |

[a] Limit of Detection, [b] Limit of quantification.

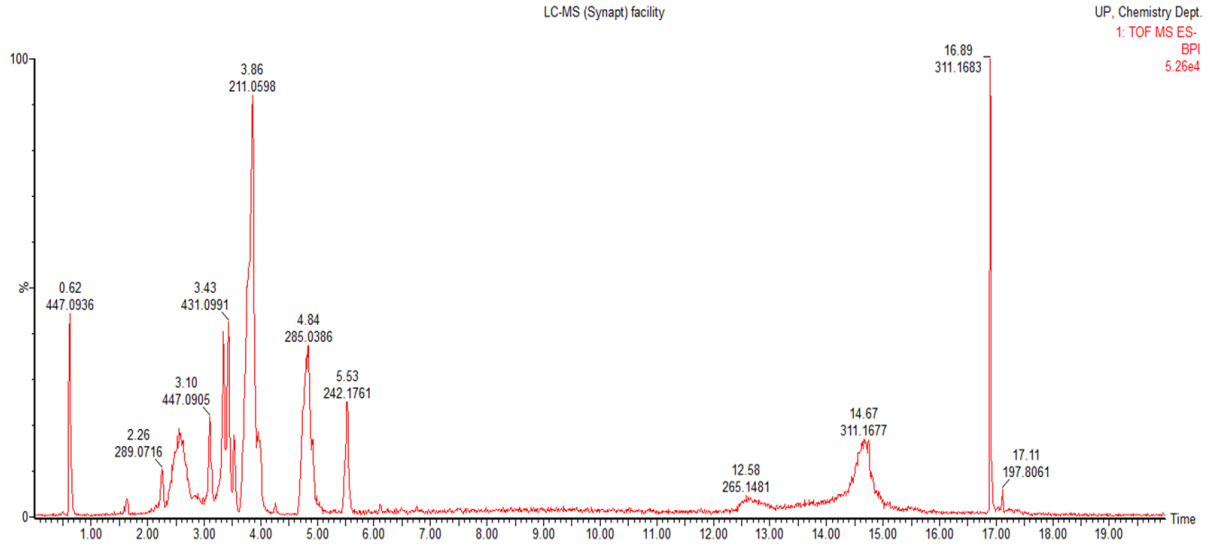



**Figure S 1:** UPLC-QTOF spectra in negative mode of the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren

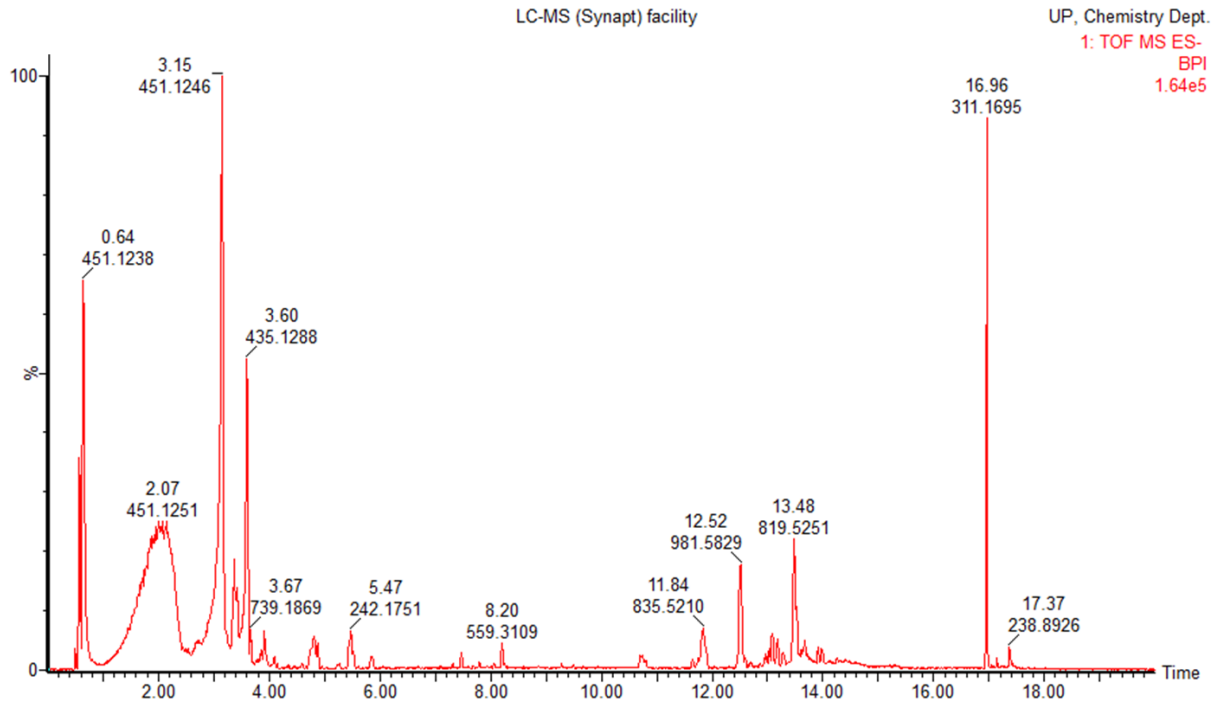
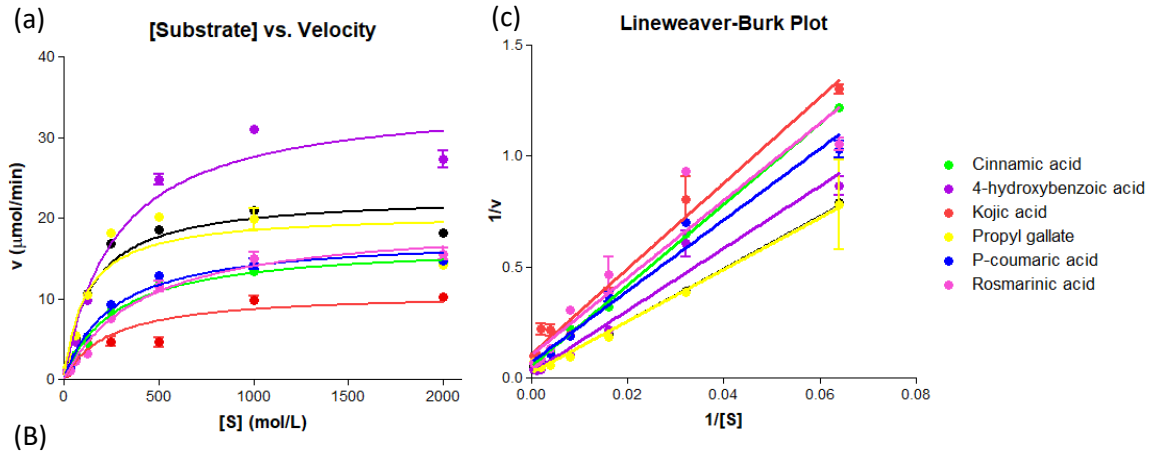



**Figure S 2:** UPLC-QTOF spectra in positive mode of the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren

# S2 - Tyrosinase Assay

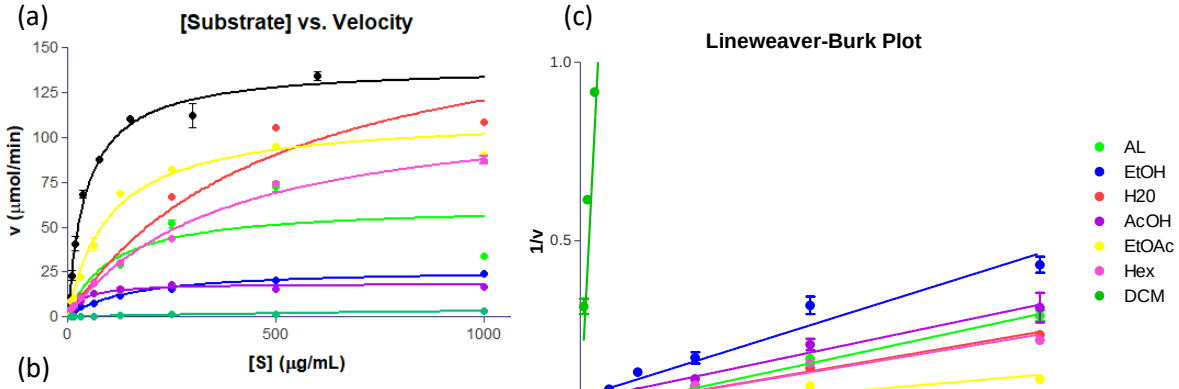
The data for the tyrosinase kinetic studies is provided in Figure S 3 to S 5.




(B)

| | v (umol/min) | Cinnamic acid | 4-Hydroxybenzoic acid | Kojic acid | Propyl gallate | p-Coumaric acid | Rosmarinic acid |
|---|---|---|---|---|---|---|---|
| VMAX | 22.82 ± 1.07 | 16.82 ± 0.37 | 35.00 ± 1.84 | 10.73 ± 1.01 | 20.56 ± 1.72 | 17.71 ± 0.66 | 19.72 ± 1.01 |
| KM | 143.8 ± 25.43 | 269.9 ± 19.51 | 267.2 ± 46.26 | 232.1 ± 64.97 | 110.80 ± 33.67 | 254.00 ± 29.91 | 401.10 ± 57.86 |

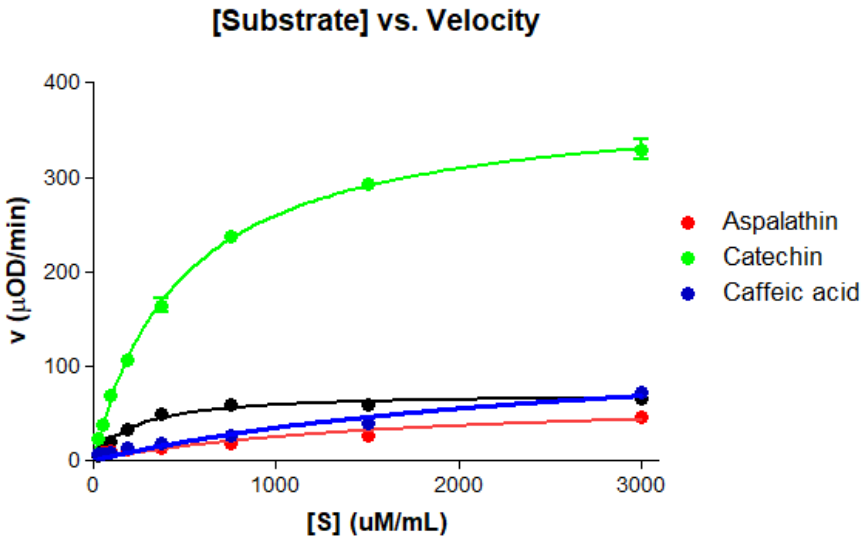
**Figure S 3:** Enzyme kinetics, namely (a) Michaelis-Menten Plot, (b) Michaelis-Menten Plot data and (c) Lineweaver-Burk Plot of the inhibitors present in *Aspalathus linearis* (Burm.f.) R.Dahlgren (https://www.graphpad.com; GraphPad Prism 5).




(b)

| | VMAX | KM |
|---|---|---|
| V (µmol/min) | 138.3 | 42.78 |
| *A. linearis* EtOH Extract (AL) | 61.86 | 104.0 |
| EtOH Fraction | 26.47 | 149.6 |
| $H_2O$ Fraction | 181.3 | 500.7 |
| AcOH | 18.37 | 31.79 |
| EtAc | 111.8 | 99.75 |
| Hex | 120.4 | 371.4 |
| DCM | 11.68 | 2732 |

**Figure S 4:** Enzyme kinetics, namely (a) Michaelis-Menten Plot, (b) Lineweaver-Burk Plot of the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren and its major fractions (https://www.graphpad.com; GraphPad Prism 5).

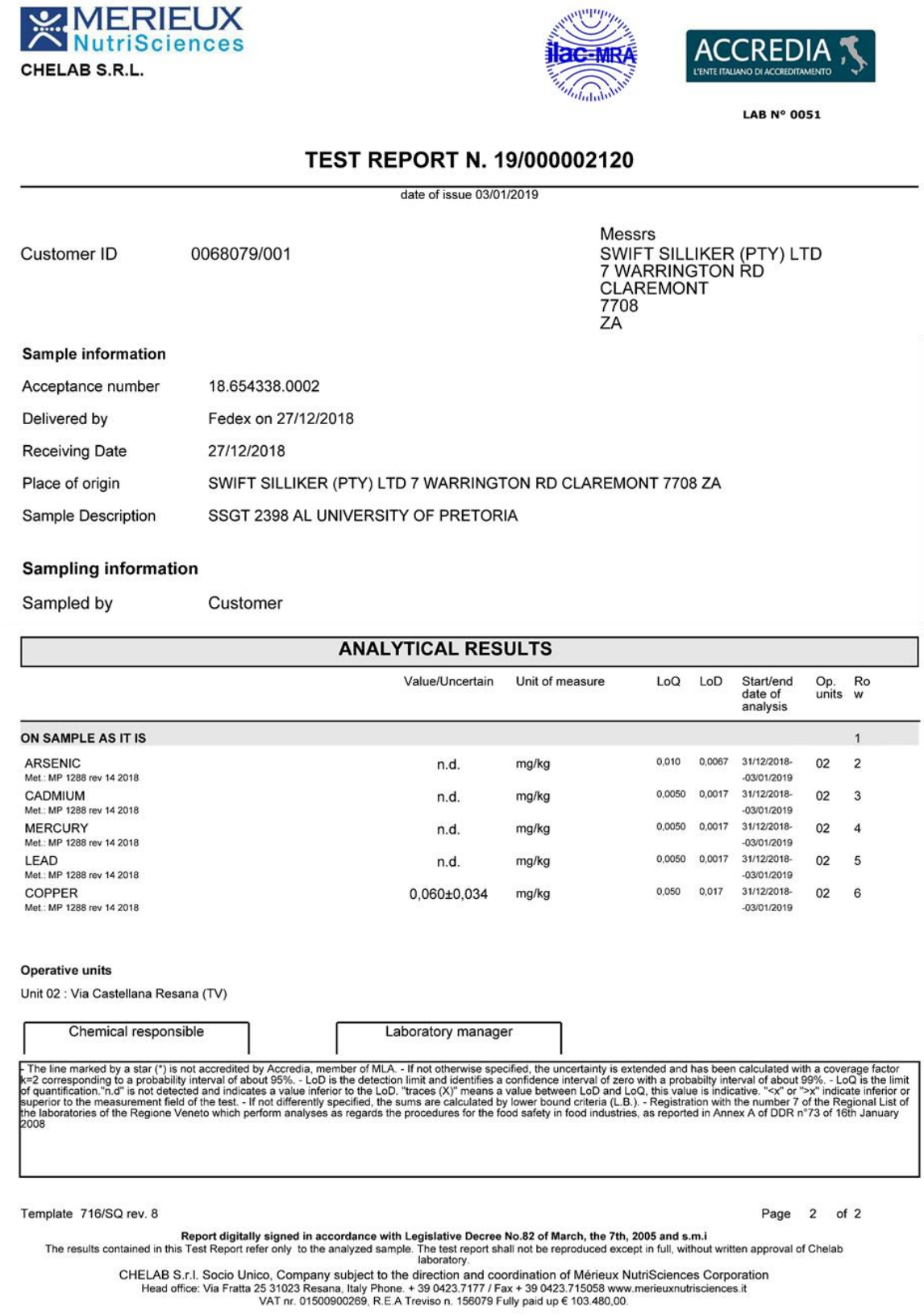



| | v (umol/min) | Aspalathin | Catechin | Caffeic acid |
|---|---|---|---|---|
| VMAX | 71.42 ± 2.61 | 68.73 ± 13.47 | 382.4 ± 7.03 | 120.9 ± 15.53 |
| KM | 211.5 ± 27.60 | 1733 ± 660.6 | 471.0 ± 26.43 | 2428 ± 553.6 |

**Figure S 5:** Michaelis-Menten Plot of the subversive substrates found in the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren. The curve represents the rate of the tyrosinase enzyme in the absence of the substrate L-dopa (https://www.graphpad.com; GraphPad Prism 5).

# S3 – Metal Analysis

The metal analysis report obtained from Merieux laboratory is provided in Figure S 6.



MERIEUX NutriSciences
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LAB N° 0051

**TEST REPORT N. 19/000002120**

date of issue 03/01/2019

Customer ID 0068079/001

Messrs
SWIFT SILLIKER (PTY) LTD
7 WARRINGTON RD
CLAREMONT
7708
ZA

**Sample information**

| | |
|---|---|
| Acceptance number | 18.654338.0002 |
| Delivered by | Fedex on 27/12/2018 |
| Receiving Date | 27/12/2018 |
| Place of origin | SWIFT SILLIKER (PTY) LTD 7 WARRINGTON RD CLAREMONT 7708 ZA |
| Sample Description | SSGT 2398 AL UNIVERSITY OF PRETORIA |

**Sampling information**

Sampled by Customer

**ANALYTICAL RESULTS**

| | Value/Uncertain | Unit of measure | LoQ | LoD | Start/end date of analysis | Op. units | Row |
|---|---|---|---|---|---|---|---|
| **ON SAMPLE AS IT IS** | | | | | | | 1 |
| ARSENIC<br>Met.: MP 1288 rev 14 2018 | n.d. | mg/kg | 0,010 | 0,0067 | 31/12/2018-03/01/2019 | 02 | 2 |
| CADMIUM<br>Met.: MP 1288 rev 14 2018 | n.d. | mg/kg | 0,0050 | 0,0017 | 31/12/2018-03/01/2019 | 02 | 3 |
| MERCURY<br>Met.: MP 1288 rev 14 2018 | n.d. | mg/kg | 0,0050 | 0,0017 | 31/12/2018-03/01/2019 | 02 | 4 |
| LEAD<br>Met.: MP 1288 rev 14 2018 | n.d. | mg/kg | 0,0050 | 0,0017 | 31/12/2018-03/01/2019 | 02 | 5 |
| COPPER<br>Met.: MP 1288 rev 14 2018 | 0,060±0,034 | mg/kg | 0,050 | 0,017 | 31/12/2018-03/01/2019 | 02 | 6 |

**Operative units**

Unit 02 : Via Castellana Resana (TV)

Chemical responsible

Laboratory manager

- The line marked by a star (*) is not accredited by Accredia, member of MLA. - If not otherwise specified, the uncertainty is extended and has been calculated with a coverage factor k=2 corresponding to a probability interval of about 95%. - LoD is the detection limit and identifies a confidence interval of zero with a probabilty interval of about 99%. - LoQ is the limit of quantification."n.d" is not detected and indicates a value inferior to the LoD. "traces (X)" means a value between LoD and LoQ, this value is indicative. "<x" or ">x" indicate inferior or superior to the measurement field of the test. - If not differently specified, the sums are calculated by lower bound criteria (L.B.). - Registration with the number 7 of the Regional List of the laboratories of the Regione Veneto which perform analyses as regards the procedures for the food safety in food industries, as reported in Annex A of DDR n°73 of 16th January 2008

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**Figure S 6:** Metal analysis report of the ethanolic extract of *Aspalathin linearis* (Burm.f.) R.Dahlgren conducted by Merieux laboratories.

# S4 - Computer modelling

The ChemSpider database IDs for the compounds are provided in Table S2. The docking of compounds **1** to **15** and kojic acid is provided in Figure S 7- S 10 for the tyrosinase and tyrosinase protein 1 structures. Please note that only the structures of aspalathin (**1**) and catechin (**3**) are provided in the manuscript.

**Table S 2** ChemSpider database IDs for the compounds docked in the 2Y9X and 5M8P protein structures

| **Compound** | **ChemSpider ID** |
|---|---|
| Aspalathin | 9457391 |
| Caffeic acid | 600426 |
| Catechin | 8711 |
| Cinnamic acid | 392447 |
| *p*-Coumaric acid | 553148 |
| Ferulic acid | 393368 |
| 4-Hydroxybenzoic acid | 132 |
| Isoquercetrin | 4444361 |
| Kojic acid | 3708 |
| Luteolin | 4444102 |
| *n*-Propyl gallate | 4778 |
| Quercetin | 12269344 |
| Rosmarinic acid | 4445104 |
| Syringic acid | 10289 |
| Vanillic acid | 8155 |
| Vitexin | 4444098 |

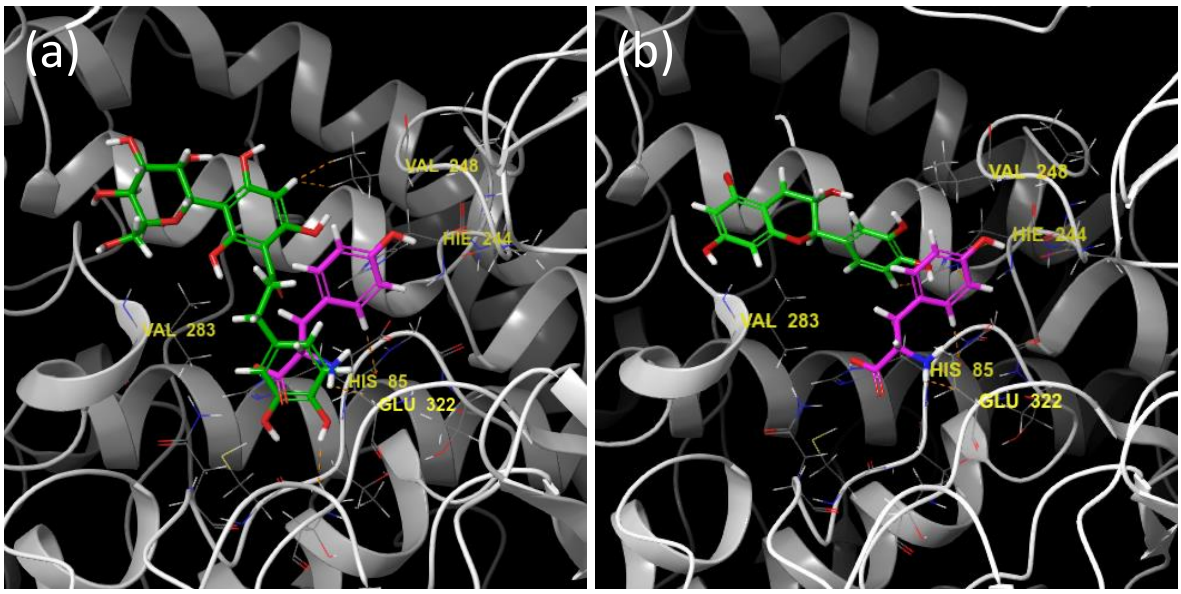



**Figure S 7**: The docking pose of (a) aspalathin (**1**) and (b) catechin (**2**) was compared to L-tyrosine and showed similar binding and alignment of the core structure to the binding site (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

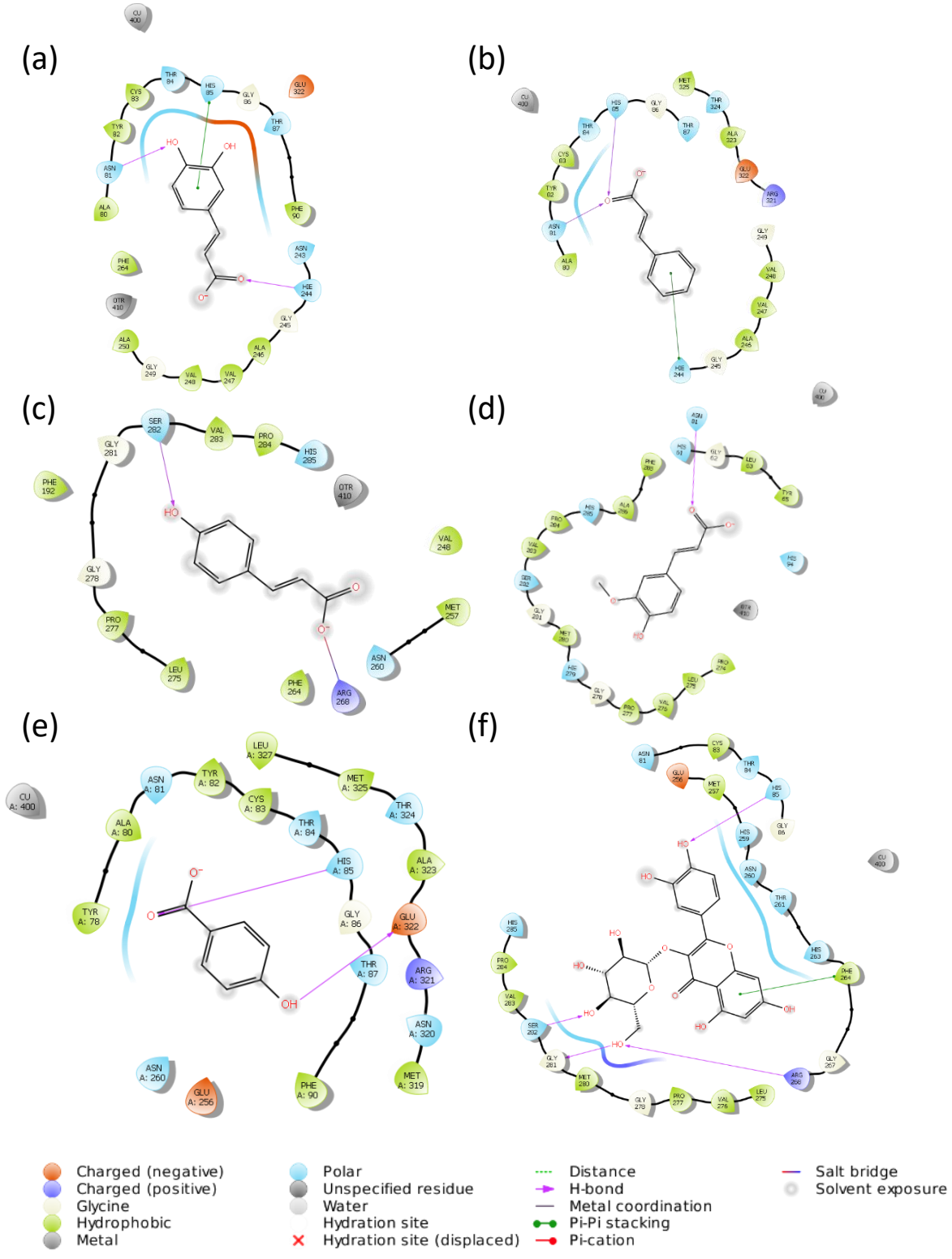



**Figure S 8:** Two-dimensional protein-ligand interactions of (a) caffeic acid, (b) cinnamic acid, (c) *p*-coumaric acid, (d) ferulic acid, (e) 4-hydroxybenzoic acid and (f) isoquercitrin with the 2Y9X tyrosinase enzyme structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

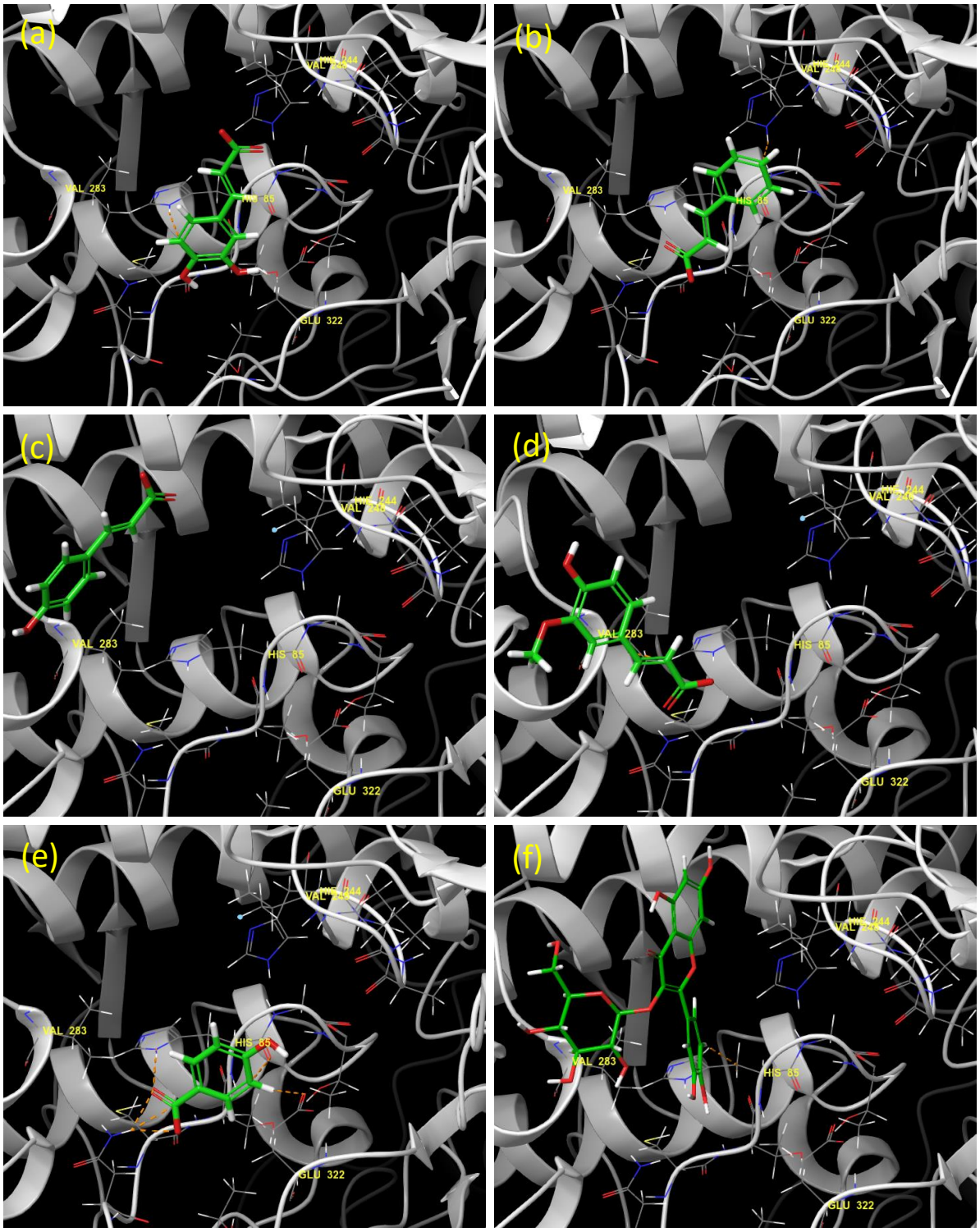



**Figure S 9:** Three-dimensional protein-ligand interactions of (a) caffeic acid, (b) cinnamic acid, (c) *p*-coumaric acid, (d) ferulic acid, (e) 4-hydroxybenzoic acid and (f) isoquercitrin with the 2Y9X tyrosinase enzyme structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

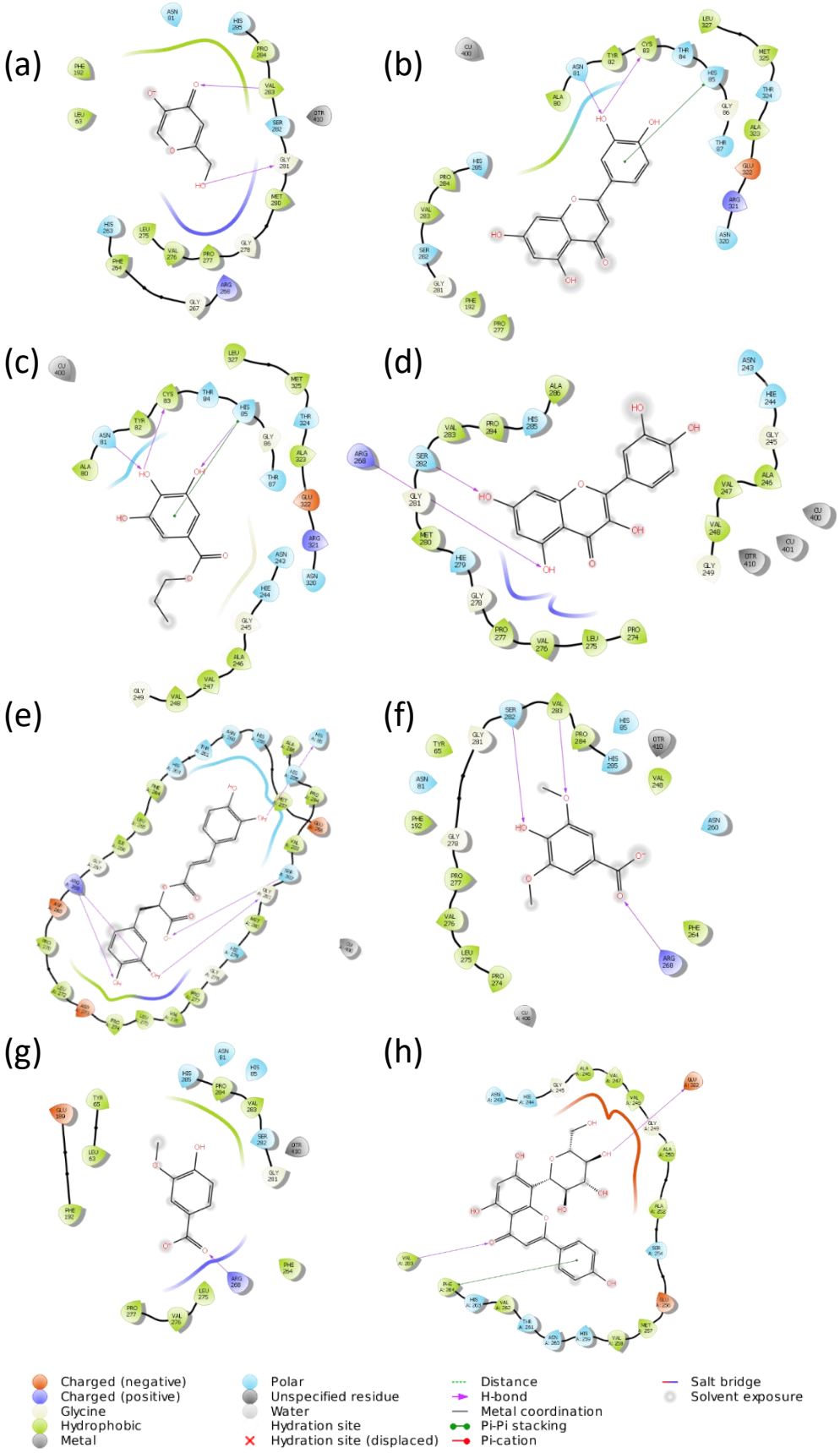



**Figure S 10:** Two-dimensional protein-ligand interactions of (a) kojic acid, (b) luteolin, (c) *n*-propyl gallate, (d) quercetin, (e) rosmarinic acid, (f) syringic acid, (g) vanillic acid and (h) vitexin with the 2Y9X tyrosinase enzyme structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

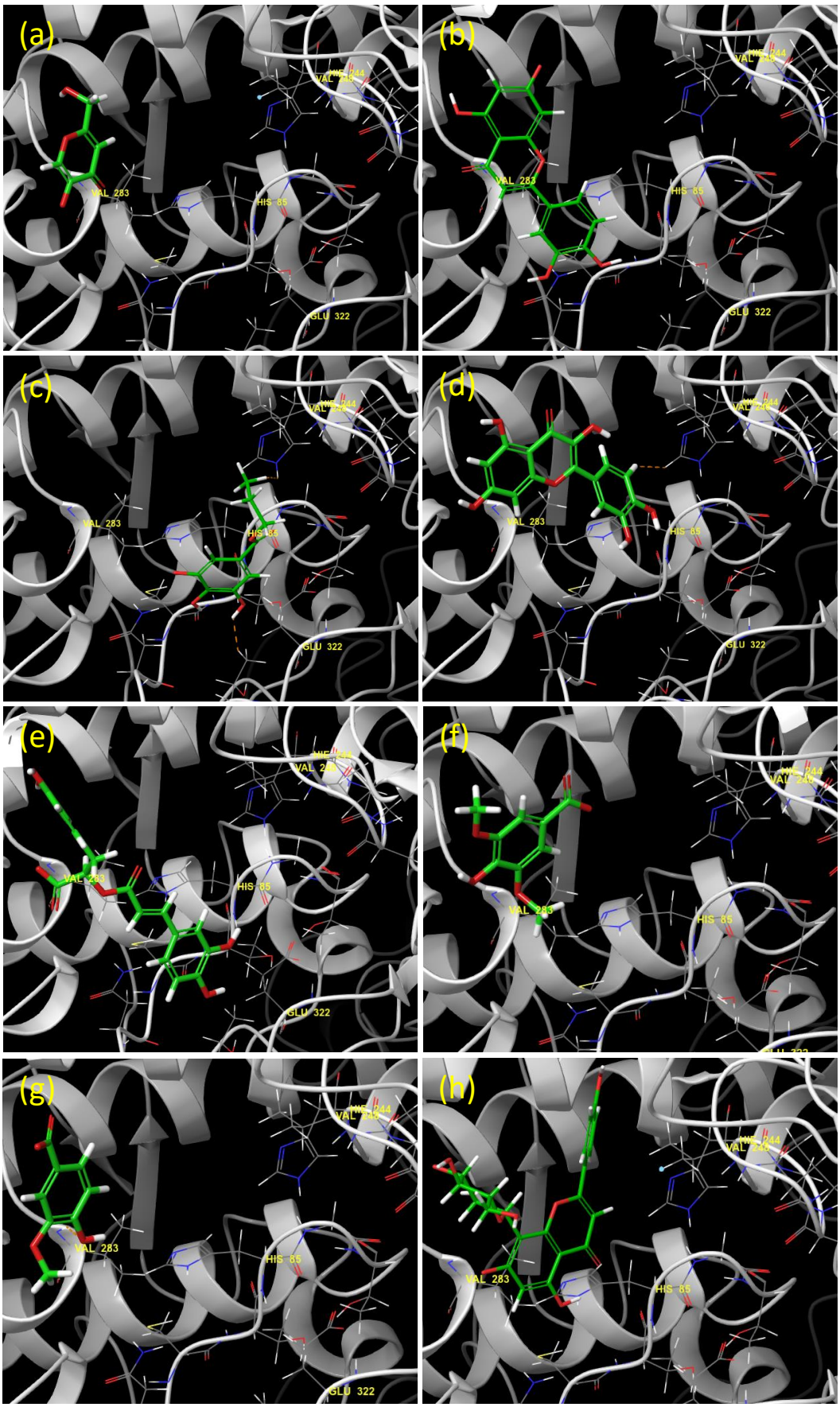



**Figure S 11:** Three-dimensional protein-ligand interactions of (a) kojic acid, (b) luteolin, (c) *n*-propyl gallate, (d) quercetin, (e) rosmarinic acid, (f) syringic acid, (g) vanillic acid and (h) vitexin with the 2Y9X tyrosinase enzyme structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

**Table S 3.** Hydrogen bond lengths between the compounds and the amino acids at the active site of the tyrosinase structure (PBD: 2Y9X) (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

| Compound | Hydrogen bond lengths 2Y9X | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **HIS 85** | **CYS 83** | **ASN 81** | **SER 282** | **GLY 281** | **ARG 268** | **HIE 244** | **GLU 322** | **VAL 283** | **ARG 250** | **ALA 246** |
| Aspalathin (**1**) | 2.21 | 1.96 | 1.99 | 2.06 | 1.91 | 2.19 | | | | | |
| Caffeic acid (**2**) | | | 1.92 | | | | 2.07 | | | | |
| Catechin (**3**) | | | | 2.01 | 2.37 | | 2.41 | | | | |
| Cinnamic acid (**4**) | 1.90 | | 2.18 | | | | | | | | |
| Ferulic acid (**5**) | | | 2.01 | | | | | | | | |
| 4-Hydroxybenzoic acid (**6**) | 1.81 | | | | | | | 2.02 | | | |
| Isoquercitrin (**7**) | 2.24 | | | 1.94 | 2.07 | 2.21 | | | | | |
| Luteolin (**8**) | | 2.23 | 2.01 | | | | | | | | |
| p-Coumaric acid (**9**) | | | | 1.90 | | | | | | | |
| *n*-Propyl gallate (**10**) | 2.31 | 2.09 | 2.09 | | | | | | | | |
| Quercetin (**11**) | | | | 1.86 | | 2.13 | | | | | |
| Rosmarinic acid (**12**) | 2.34 | | | 2.06 | 1.81 | | | | | 2.34 | |
| Syringic acid (**13**) | | | | 2.09 | | 2.05 | | | 2.10 | | |
| Vanillic acid (**14**) | | | | | | 1.63 | | | | | |
| Vitexin (**15**) | | | | | | | | 1.97 | 2.73 | | |
| Kojic acid | | | | | 1.81 | | | | 2.22 | | |
| L-tyrosine | 2.04 | | 2.73 | | | | | 2.52 | | | 2.11 |

Short Long

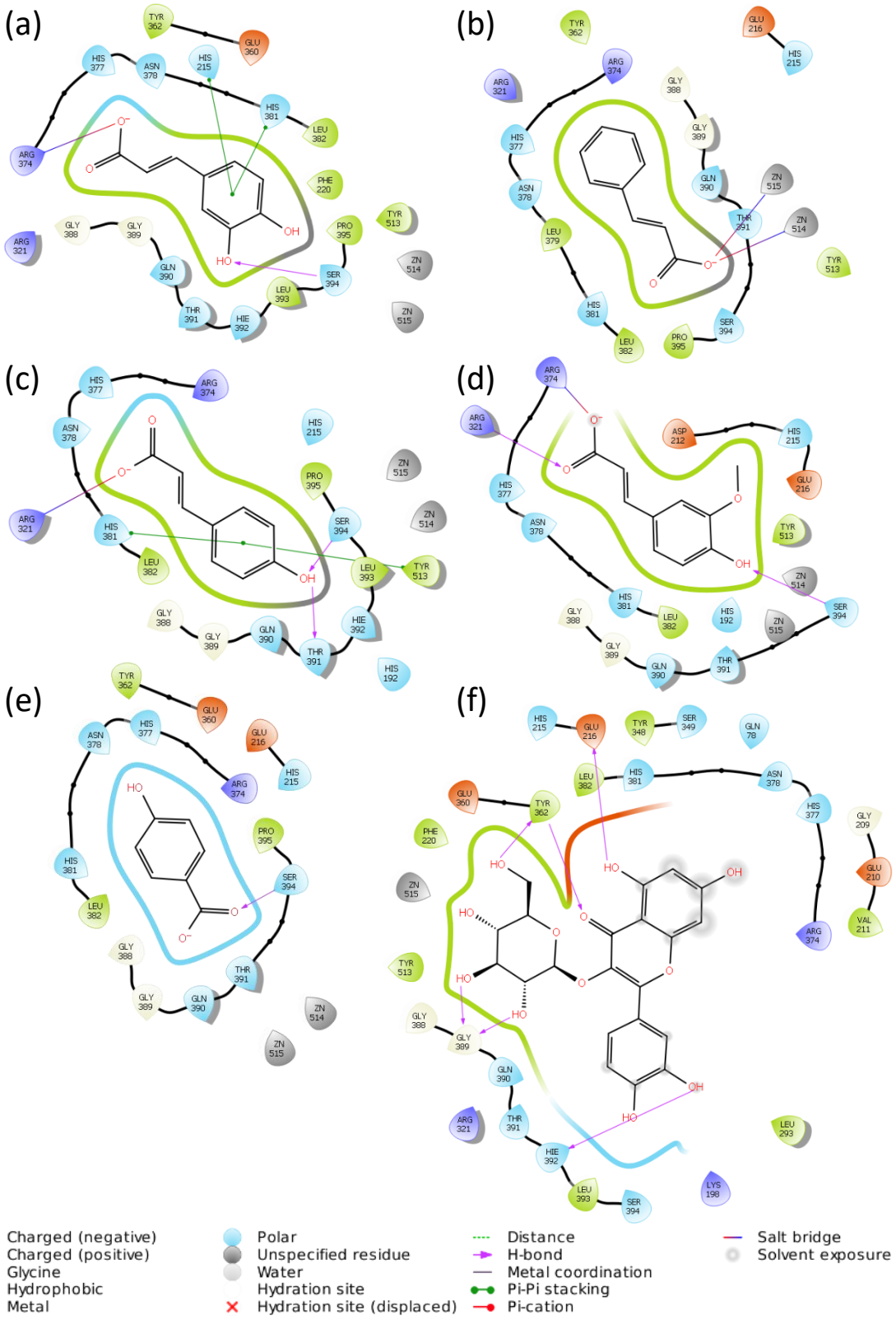



**Figure S 12:** Two-dimensional protein-ligand interactions of (a) caffeic acid, (b) cinnamic acid, (c) *p*-coumaric acid, (d) ferulic acid, (e) 4-hydroxybenzoic acid and (f) isoquercitrin with the 5M8P tyrosinase protein 1 structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

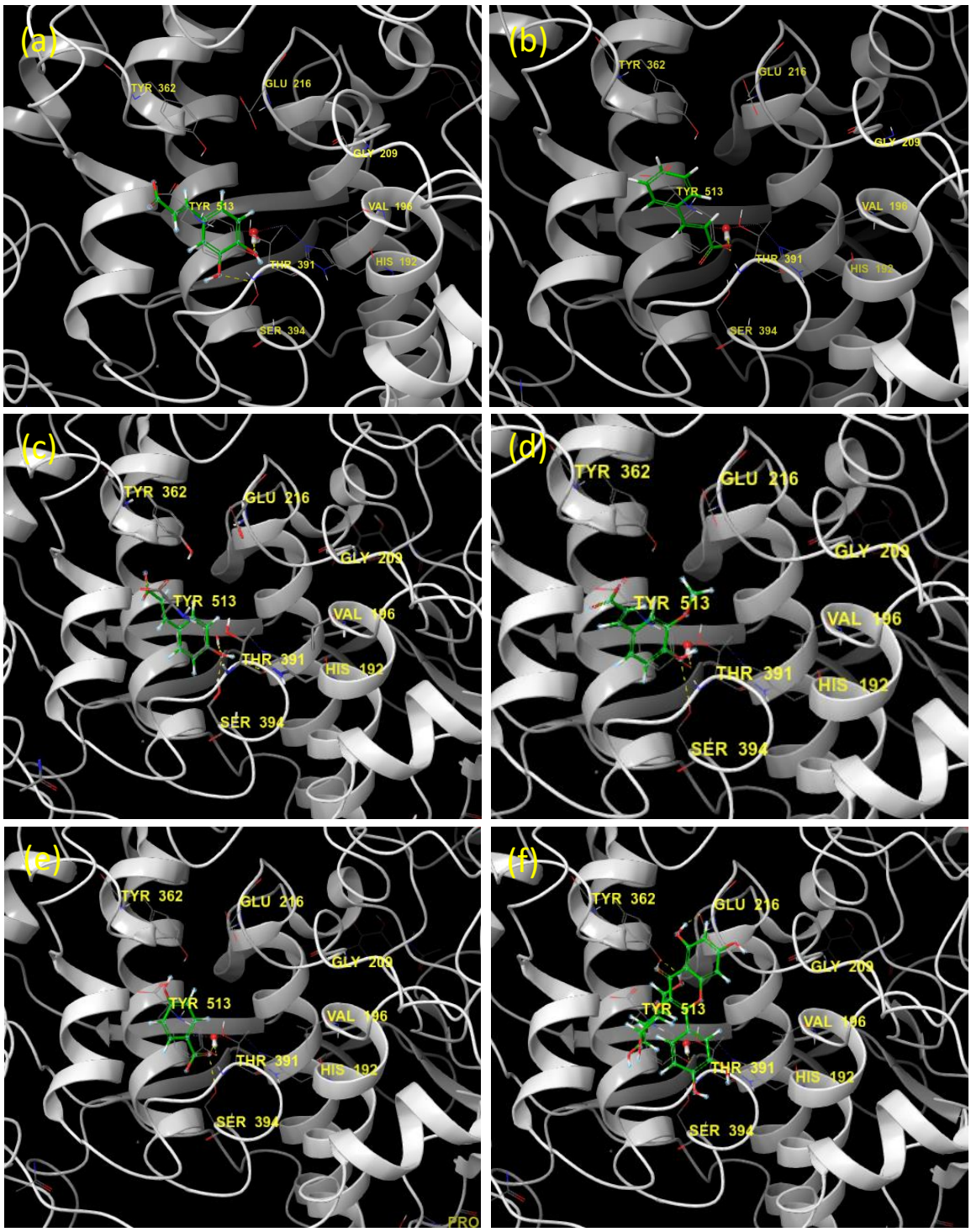



**Figure S 13:** Three-dimensional protein-ligand interactions of (a) caffeic acid, (b) cinnamic acid, (c) *p*-coumaric acid, (d) ferulic acid, (e) 4-hydroxybenzoic acid and (f) isoquercetrin with the 5M8P tyrosinase protein 1 structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

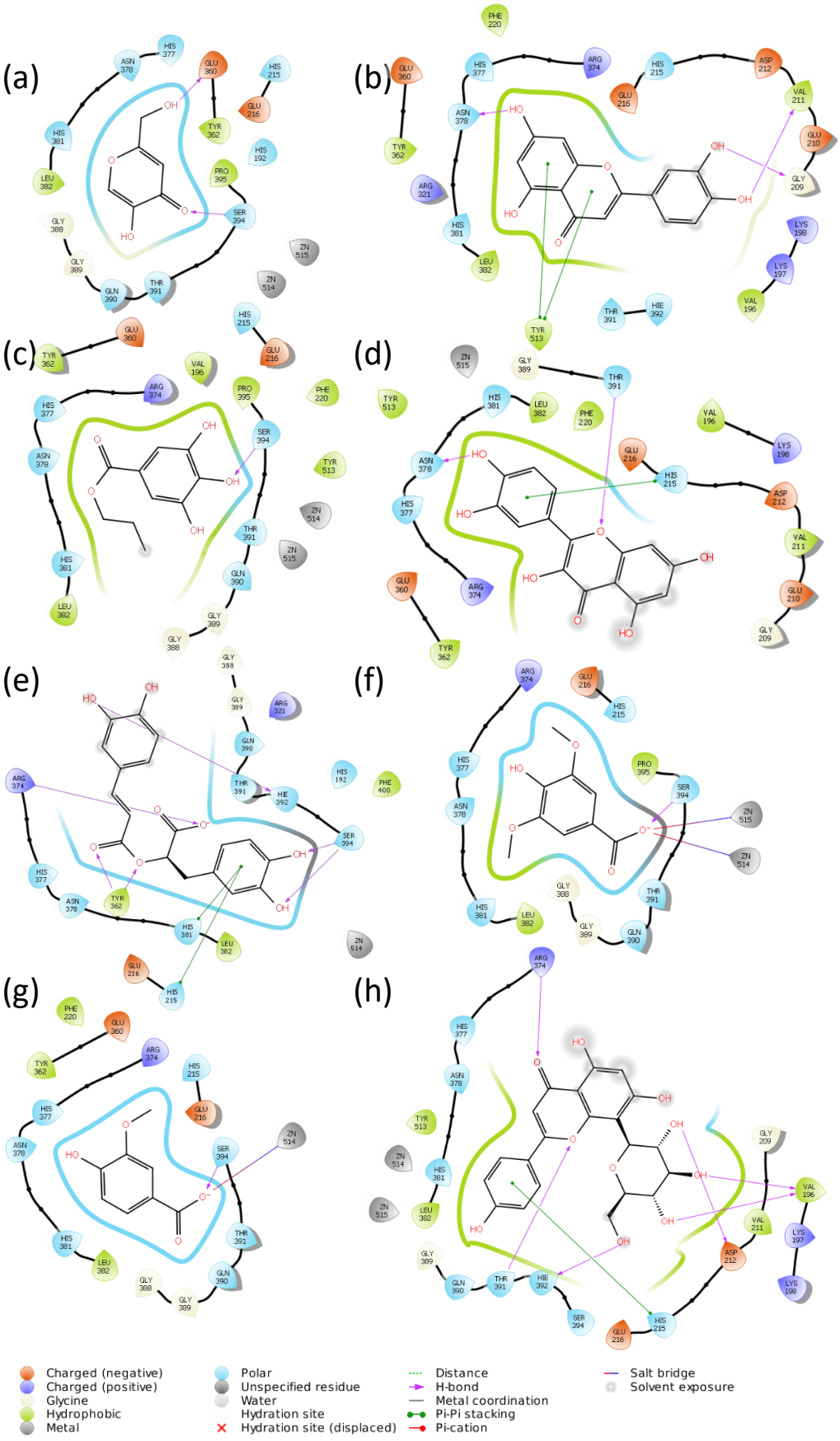



**Figure S 14:** Two-dimensional protein-ligand interactions of (a) kojic acid, (b) luteolin, (c) *n*-propyl gallate, (d) quercetin, (e) rosmarinic acid, (f) syringic acid, (g) vanillic acid and (h) vitexin with the 5M8P tyrosinase protein 1 structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

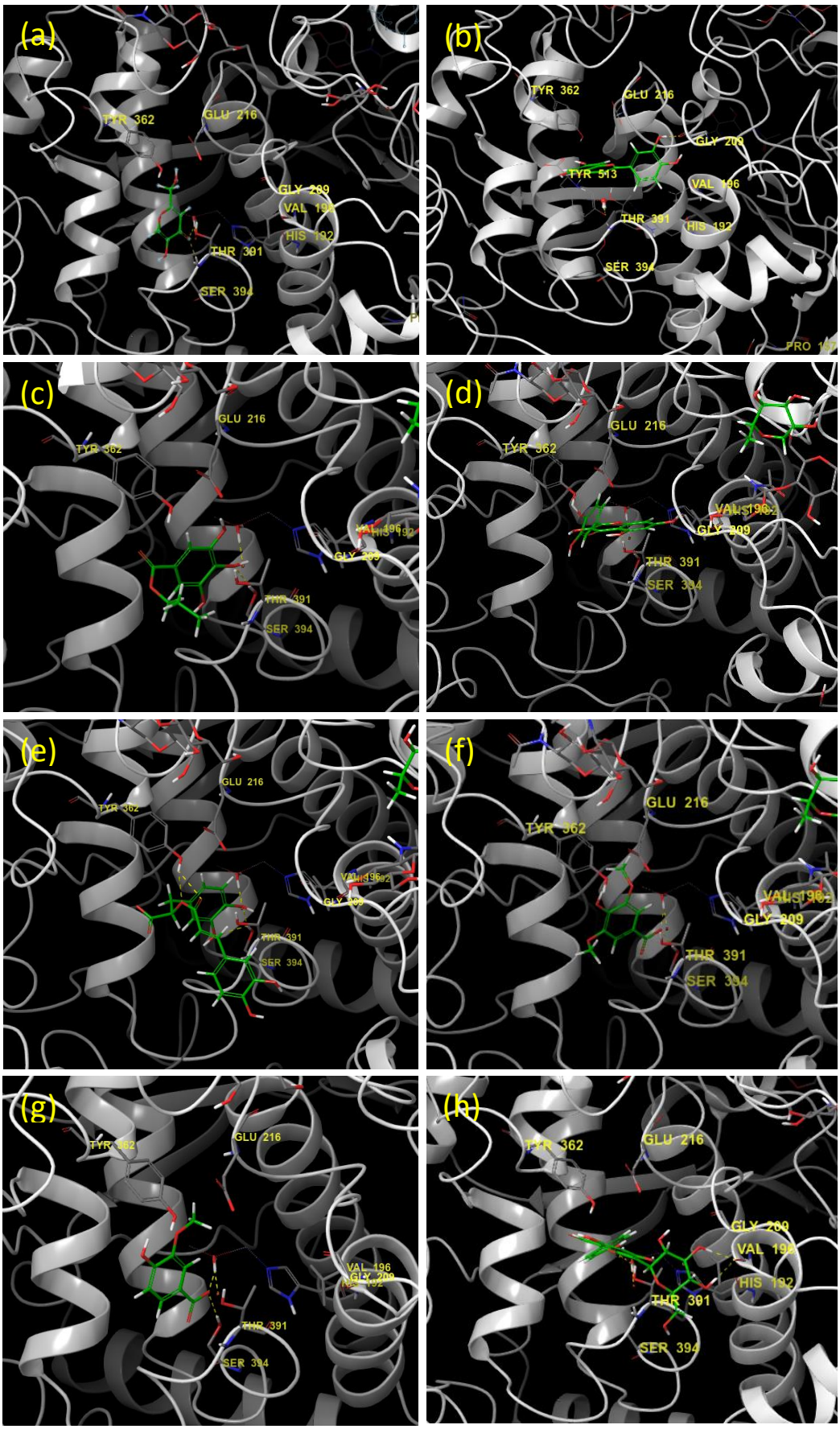



**Figure S 15:** Three-dimensional protein-ligand interactions of (a) kojic acid, (b) luteolin, (c) *n*-propyl gallate, (d) quercetin, (e) rosmarinic acid, (f) syringic acid, (g) vanillic acid and (h) vitexin with the 5M8P tyrosinase protein 1 structure (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

**Table S 4.** Hydrogen bond lengths between the compounds and the amino acids at the active site of the tyrosinase-related protein 1 structure (PBD: 5M8P) (https://www.schrodinger.com/maestro; version 12.1.013, release 2019-3).

| Compounds | Hydrogen bond lengths 5M8P | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **GLY 209** | **GLU 216** | **TYR 362** | **GLY 388** | **SER 394** | **ASN 378** | **VAL 196** | **ARG 321** | **GLY 389** | **HIE 392** | **VAL 211** | **THR 391** | **ARG 374** | **VAL 196** | **ASP 212** | **GLU 360** |
| Aspalathin (**1**) | 1.95 | 2.03 | 2.14 | 2.42 | 2.27 | | | | | | | | | | | |
| Caffeic acid (**2**) | | | | | 2.67 | | | | | | | | | | | |
| Catechin (**3**) | | | | | | 1.86 | 1.80 | | | | | | | | | |
| Cinnamic acid (**4**) | | | | | | | | | | | | | | | | |
| Ferulic acid (**5**) | | | | | 2.42 | | | 2.33 | | | | | | | | |
| 4-Hydroxybenzoic acid (**6**) | | | | | 1.97 | | | | | | | | | | | |
| Isoquercitrin (**7**) | | 1.79 | 1.79 | | | | | | 1.89 | 2.39 | | | | | | |
| Luteolin (**8**) | 1.91 | | | | | 1.89 | | | | | 2.06 | | | | | |
| p-Coumaric acid (**9**) | | | | | 1.79 | | | | | | | 2.78 | | | | |
| *n*-Propyl gallate (**10**) | | | | | 2.18 | | | | | | | | | | | |
| Quercetin (**11**) | | | | | | 2.00 | | | | | | 2.11 | | | | |
| Rosmarinic acid (**12**) | | | 2.43 | | 1.94 | | | | | 2.25 | | | 1.94 | | | |
| Syringic acid (**13**) | | | | | 1.82 | | | | | | | | | | | |
| Vanillic acid (**14**) | | | | | 2.05 | | | | | | | | | | | |
| Vitexin (**15**) | | | | | | | | | | 2.21 | | 2.51 | 1.96 | 1.86 | 2.38 | |
| Kojic acid | | | | | 1.96 | | | | | | | | | | | 2.34 |

## S5 - Pearson correlation

The data obtained using the Pearson correlation is provided in Table S 5 and Figure S 16.

**Table S 5.** Pearson correlation, assuming a Gaussian distribution between the tyrosinase activity and the docking scores for the tyrosinase structure (PBD: 2Y9X) and for the tyrosinase related protein 1 structure (PBD: 5M8P) (https://www.graphpad.com; GraphPad Prism 5)

| **Pearson Correlation** | | | |
|---|---|---|---|
| | **Correlation between Tyrosinase activity and 2Y9X docking score** | **Correlation between Tyrosinase activity and 5M8P docking score** | **Correlation between 2Y9X and 5M8P docking scores** |
| Number of XY Pairs | 16 | 16 | 16 |
| Pearson r | -0.5696 | -0.4719 | 0.7618 |
| 95% confidence interval | -0.8308 to -0.1028 | -0.7842 to 0.03119 | 0.4275 to 0.9128 |
| P value (two-tailed) | 0.0213 | 0.065 | 0.0006 |
| P value summary | * | ns | *** |
| Is the correlation significant? (alpha=0.05) | Yes | No | Yes |
| R squared | 0.3244 | 0.2227 | 0.5804 |

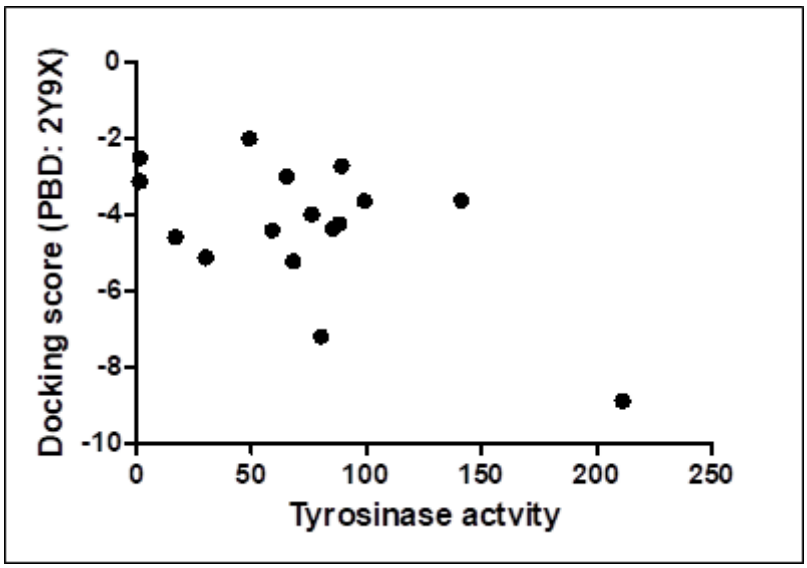


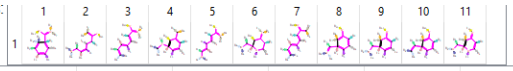

**Figure S 16:** Pearson correlation, assuming Gaussian distribution, between the tyrosinase activity and the docking scores for the tyrosinase structure (PBD: 2Y9X) (https://www.graphpad.com; GraphPad Prism 5).

# S6 - Quantitative Structure–Activity Relationship Model

The data obtained using the Quantitative Structure–Activity Relationship Model is provided in Table S 6 to S 9.

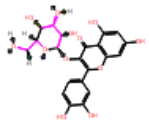
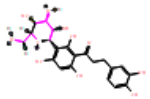
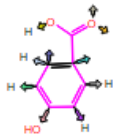
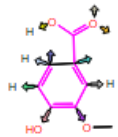
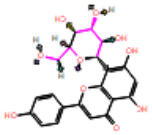
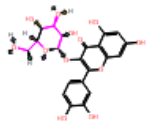
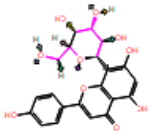
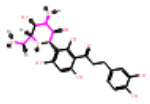
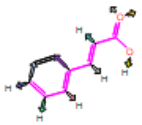
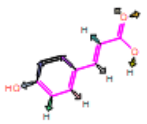
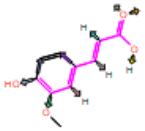
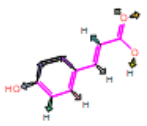
**Table S 6.** Structure Activity Relationship Table for the compounds identified in the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren (https://www.schrodinger.com/canvas; version 4.1.013, release 2019-3).

Cores:



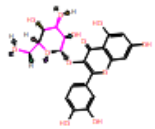
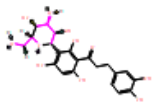
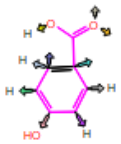
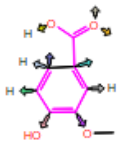
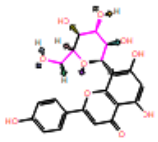
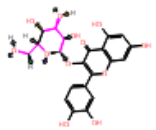
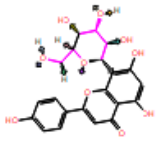
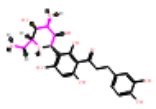
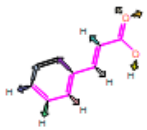
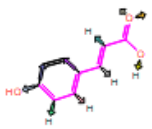
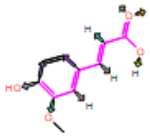
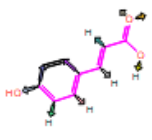

| | Input # | Structure Name | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Vanillic acid |  |  |  |  |  |  |  |  |  |  |
| 2 | 2 | Quercetin |  |  |  |  |  |  |  |  |  |  |
| 3 | 3 | P-Coumaric Acid |  |  |  |  |  |  |  |  |  |  |
| 4 | 4 | Syringic acid |  |  |  |  |  |  |  |  |  |  |
| 5 | 5 | Cinnamic Acid |  |  |  |  |  |  |  |  |  |  |
| 6 | 6 | Caffeic Acid |  |  |  |  |  |  |  |  |  |  |
| 7 | 7 | Aspalathin |  |  |  |  |  |  |  |  |  |  |
| 8 | 8 | Luteolin |  |  |  |  |  |  |  |  |  |  |
| 9 | 9 | Ferulic Acid |  |  |  |  |  |  |  |  |  |  |
| 10 | 10 | Propyl Gallate |  |  |  |  |  |  |  |  |  |  |
| 11 | 11 | (=)-catechin |  |  |  |  |  |  |  |  |  |  |
| 12 | 12 | Rosmarinic Acid |  |  |  |  |  |  |  |  |  |  |
| 13 | 13 | Isoquercitrin |  |  |  |  |  |  |  |  |  |  |
| 14 | 14 | 4-Hydroxybenz... |  |  |  |  |  |  |  |  |  |  |
| 15 | 15 | Vitexin |  |  |  |  |  |  |  |  |  |  |
| 16 | 16 | Protocatechuic... |  |  |  |  |  |  |  |  |  |  |
| 17 | 17 | Kojic acid |  |  |  |  |  |  |  |  |  |  |

**Table S 7.** The tyrosinase property activity cliffs of the R-group analysis of the compounds identified in the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren (https://www.schrodinger.com/canvas; version 4.1.013, release 2019-3).

| | Deviation | Structure 1 | Structure 2 | Δ Fingerprint | \| Δ Property \| |
|---|---|---|---|---|---|
| 1 | 1.23 |  Isoquercitrin |  Aspalathin | 10.0 | 131.0 |
| 2 | -1.19 | 89.00  4-Hydroxybenzoic Acid | 88.00  Vanillic acid | 5.0 | 1.0 |
| 3 | -1.9 | 68.00  Vitexin | 80.00  Isoquercitrin | 10.0 | 12.0 |
| 4 | 1.29 | 68.00  Vitexin | 211.00  Aspalathin | 8.94 | 143.0 |
| 5 | -1.16 | 49.00  Cinnamic Acid | 1.50  P-Coumaric Acid | 4.47 | 47.5 |
| 6 | -0.66 | 65.00  Ferulic Acid | 1.50  P-Coumaric Acid | 5.83 | 63.5 |

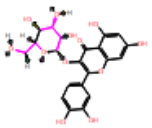
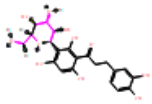
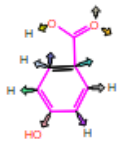
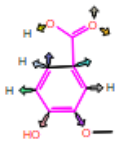
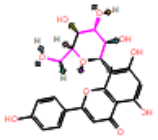
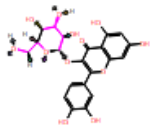
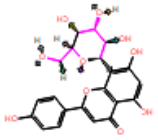
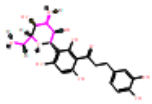
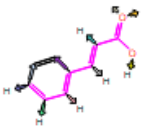
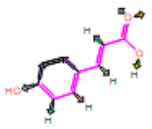
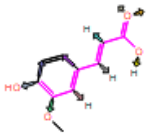
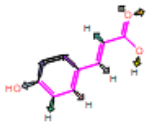
**Deviation:**
greater than 4
between 2 and 4
between -2 and 2
between -4 and -2
less than -4

**Table S 8.** The docking score for the tyrosinase structure (PBD: 2Y9X) property activity cliffs of the R-group analysis of the compounds identified in the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren (https://www.schrodinger.com/canvas; version 4.1.013, release 2019-3).

| | Deviation | Structure 1 | Structure 2 | Δ Fingerprint | \| Δ Property \| |
|---|---|---|---|---|---|
| 1 | 1.39 | -7.19  Isoquercitrin | -8.88  Aspalathin | 11.27 | 1.69 |
| 2 | -1.21 | -2.71  4-Hydroxybenzoic Acid | -4.24  Vanillic acid | 5.29 | 1.53 |
| 3 | 0.99 | -5.21  Vitexin | -7.19  Isoquercitrin | 10.86 | 1.98 |
| 4 | 1.82 | -5.21  Vitexin | -8.88  Aspalathin | 10.82 | 3.67 |
| 5 | -1.15 | -2.00  Cinnamic Acid | -2.51  P-Coumaric Acid | 4.58 | 0.51 |
| 6 | -0.99 | -2.99  | -2.51  | 6.0 | 0.48 |

**Deviation:**
greater than 4
between 2 and 4
between -2 and 2
between -4 and -2
less than -4

**Table S 9.** The docking score for the tyrosinase related protein 1 structure (PBD: 5M8P) property activity cliffs of the R-group analysis of the compounds identified in the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren (https://www.schrodinger.com/canvas; version 4.1.013, release 2019-3).

| | Deviation | Structure 1 | Structure 2 | Δ Fingerprint | \| Δ Property \| |
|---|---|---|---|---|---|
| 1 | 1.19 |  Isoquercitrin |  Aspalathin | 11.27 | 200.0 |
| 2 | 1.49 | 258.10  4-Hydroxybenzoic Acid | 0.0  Vanillic acid | 5.29 | 258.1 |
| 3 | -1.53 | 200.00  Vitexin | 200.00  Isoquercitrin | 10.86 | 0.0 |
| 4 | 1.08 | 200.00  Vitexin | 0.0  Aspalathin | 10.82 | 200.0 |
| 5 | -1.14 | 139.80  Cinnamic Acid | 25.03  P-Coumaric Acid | 4.58 | 114.77 |
| 6 | -1.25 | 0.0  Ferulic Acid | 25.03  P-Coumaric Acid | 6.0 | 25.03 |

**Deviation:**
greater than 4
between 2 and 4
between -2 and 2
between -4 and -2
less than -4

# S7 - Antiproliferation Assay

The data obtained for the antiproliferation assay is provided in Table S 10.

**Table S 10.** Sigmoidal dose response curve analysis of the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren (AL), its fractions and the compounds aspalathin and catechin (https://www.graphpad.com; GraphPad Prism 5)

| **Sigmoidal dose-response (variable slope)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Best-fit values** | **AL** | **DCM** | **Acetic** | **$H_2O$** | **EtAc** | **EtOH** | **Hexane** | **Aspalathin** | **Catechin** |
| Bottom | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Top | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $LogEC_{50}$ | 2.22 | 2.10 | 2.32 | 2.56 | 2.37 | 2.36 | 2.09 | 2.34 | 2.22 |
| HillSlope | -2.18 | -2.67 | -1.76 | -1.93 | -4.45 | -2.19 | -2.18 | -5.86 | -3.06 |
| $EC_{50}$ | 164.80 | 126.50 | 208.50 | 359.60 | 231.70 | 230.70 | 123.20 | 217.70 | 166.20 |
| **Std. Error** | | | | | | | | | |
| $LogEC_{50}$ | 0.05 | 0.06 | 0.03 | 0.06 | 0.04 | 0.04 | 0.07 | 0.08 | 0.03 |
| HillSlope | 0.48 | 0.83 | 0.23 | 0.53 | 2.08 | 0.37 | 0.67 | 11.81 | 0.71 |
| **95% CI (profile likelihood)** | | | | | | | | | |
| $LogEC_{50}$ | 2.12 to 2.32 | 1.99 to 2.22 | 2.25 to 2.39 | 2.44 to 2.704 | 2.28 to 2.44 | 2.28 to 2.44 | 1.95 to 2.24 | 2.16 to 2.52 | 2.15 to 2.29 |
| HillSlope | -3.36 to -1.51 | -6.36 to -1.69 | -2.33 to -1.34 | -3.34 to -1.19 | 6.76 to -2.31 | -3.42 to -1.51 | -4.33 to -1.34 | -32.17 to 20.46 | -4.63 to -1.48 |
| EC50 | 130.30 to 208.80 | 96.55 to 166.60 | 177 to 245.50 | 274.80 to 505.60 | 188.50 to 275.40 | 192 to 274 | 88.26 to 174.40 | 144.50 to 328.00 | 140.30 to 196.80 |
| **Goodness of Fit** | | | | | | | | | |
| Degrees of Freedom | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 10 | 10 |
| R square | 0.89 | 0.87 | 0.92 | 0.76 | 0.86 | 0.90 | 0.82 | 0.67 | 0.89 |
| Absolute Sum of Squares | 4313 | 6943 | 1671 | 4735 | 5569 | 2349 | 8685 | 2130 | 1203 |
| Sy.x | 14.00 | 17.77 | 8.71 | 14.67 | 15.91 | 10.33 | 19.87 | 14.60 | 10.97 |
| **Constraints** | | | | | | | | | |
| Bottom | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Top | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Number of points analysed | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 12 | 12 |

# S8 - Masson Fontana Assay

The data obtained for the Masson Fontana assay is provided in Table S 11.

**Table S 11.** One-way analysis of variance and the post-hoc statistical analyses (Tukey's multiple comparison tests) of the number of melanin produced in melanocytes (UCT-Mel1) treated with the ethanolic extract of *Aspalathus linearis* (Burm.f.) R.Dahlgren (AL), its fractions and the compounds aspalathin and catechin vs the untreated (negative) melanocytes (https://www.graphpad.com; GraphPad Prism 5)

| **One-way analysis of variance** | | | | | |
|---|---|---|---|---|---|
| P value | P<0.0001 | | | | |
| P value summary | *** | | | | |
| Are means signif. different? (P < 0.05) | Yes | | | | |
| Number of groups | 11 | | | | |
| F | 7.028 | | | | |
| R squared | 0.5273 | | | | |
| **ANOVA Table** | **SS** | **df** | **MS** | | |
| Treatment (between columns) | 1511 | 10 | 151.1 | | |
| Residual (within columns) | 1355 | 63 | 21.5 | | |
| Total | 2866 | 73 | | | |
| **Dunnett's Multiple Comparison Test** | **Mean Diff.** | **q** | **Significant? P < 0.05?** | **Summary** | **95% CI of diff** |
| Negative vs aMSH 4.5uM | -7.413 | 2.841 | Yes | * | -14.71 to -0.1129 |
| Negative vs aMSH 9uM | -9.156 | 4.51 | Yes | *** | -14.83 to -3.476 |
| Negative vs aMSH 18uM | -15.06 | 5.771 | Yes | *** | -22.36 to -7.760 |
| Negative vs AL | -9.23 | 4.875 | Yes | *** | -14.53 to -3.934 |
| Negative vs AcOH | -9.572 | 4.273 | Yes | *** | -15.84 to -3.306 |
| Negative vs EtOAc | -9.107 | 3.49 | Yes | ** | -16.41 to -1.807 |
| Negative vs EtOH | -11.17 | 4.663 | Yes | *** | -17.86 to -4.467 |
| Negative vs $H_2O$ | -8.718 | 4.107 | Yes | ** | -14.66 to -2.780 |
| Negative vs Aspalathin | -8.382 | 3.742 | Yes | ** | -14.65 to -2.116 |
| Negative vs Catechin | -15.08 | 6.297 | Yes | *** | -21.78 to -8.380 |