Molecular docking analysis of *Cissus rotundifolia* constituents as Tyrosinase inhibitors

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Article History: Received: 02.10.2022 **Revised:** 23.12.2022 **Accepted:** 17.01.2023

ABSTRACT

Introduction and Aim: *Cissus rotundifolia* has been reported to possess various pharmacological activities. In the current study, we aimed to evaluate 36 selected constituents of *Cissus rotundifolia* as tyrosinase inhibitors using *in silico* approach.

Methods: The thirty six selected constituents of *Cissus rotundifolia* was evaluated on the docking behaviour of tyrosinase by using the patchdock method.

Results: In the present study, twelve ligands belong to flavonoid and flavonoid glycoside classes, respectively. The docking study revealed that quercetin-3-*O*-galactoside exhibited the highest (ACE) atomic contact energy (-145.70 kcal/mol) and on the other hand epiafzelechin showed the lowest binding energy (-3.45 kcal/mol) with the target enzyme tyrosinase. Interestingly one ligand (quercetin-3-*O*-galactoside) was shown to have an interaction with the His 178 amino acid residue of the tyrosinase.

Conclusion: All the thirty six selected constituents of *Cissus rotundifolia* have shown to dock with the target enzyme tyrosinase. However, seven ligands namely, aureusidin, catechin gallate, 5-Hydroxy-6, 7, 8, 30, 40 – pentamethoxyflavone, pinobanksin, quercitrin, quinic acid, and vitexin-7-*O*-(6"-*p*- coumaroyl) glucoside have shown poor (ACE) atomic contact energy values. These results provide new insight in understanding their inhibitory activities and pave the way for further investigation of these 36 selected *Cissus rotundifolia* constituents as possible inhibitors against tyrosinase.

Keywords: *Cissus rotundifolia*; docking; quercetin-3-*O*-galactoside; epiafzelechin; tyrosinase.

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INTRODUCTION

Cissus rotundifolia is a perennial, evergreen, climber and is a species of Cissus belonging to the family of Vitaceae (grape family). It is commonly known as Peruvian Grape Ivy, Venezuelan Tree bine (1) and locally/vernacular known (in Tamil- South India) as "Ilai pirandai" (2), (in southern part of Saudi Arabia) as "Algalaf" (1), (in Yemen) as "Halas (or) Alfaq" (3). It is native to Africa and the Arabian Peninsula. In the year 2013, it is recorded for the first time from the Peninsular India (4). Ansarali and co-workers (2018) had extracted the genomic DNA from leaf of *C. rotundifolia* without using liquid nitrogen (5). *C. rotundifolia* is largely consumed by local people as a green leafy vegetable: the leaves of *C. rotundifolia* have been cooked to prepare a wide variety of dishes with good nutritional value (1). C. *rotundifolia* has been traditional used to treat burns, skin diseases, liver, and gastrointestinal (GI) disorders (6). C. *rotundifolia* has been reported to possess various biological activities such as i) anti-diabetic, ii) anti-fertility, iii) anti-hyperlipidemic, iv) anti-malarial, v) anti-osteoporotic, and vi) anti-parasitic activities. Recently, *C. rotundifolia* 11 selected constituents have been reported as potential human neutrophil elastase, matrix metalloproteinases (MMP 2 and 9) and tyrosinase inhibitors using *in silico* study (7).

Tyrosinase is a copper-containing enzyme which initiates melanogenesis via by hydroxlating L-Tyrosine to form L-DOPA (3, 4-dihydroxyphenyl-alanine) and further oxidizing L-DOPA to Dopaquinone (8). Tyosinase is a key regulatory enzyme in melanin biosynthesis pathway and the melanin content in cell correlates well with the catalytic activity and in tyrosinase protein level. Tyrosinase has been served as immuno-marker for the diagnosis of melanocytic lesions and differential diagnosis of metastatic malignant melanoma (9). Recently, the tyrosinase has been reported to involve in neurodegenerative diseases like Huntingtin's and Parkinson's disease (10). Most of the skin-lightening agents notably phytochemicals, have been reported to inhibit the tyrosinase activity. The above-mentioned background prompts us to carry out the present work, on thirty six selected constituents of *Cissus rotundifolia* namely aureusidin, avicularin, calycosin, catechin, gallate, delphinidin, epiafzelechin, epicatechin, epigallocatechin, gallocatechin, .isohemiphloin, isoorientin-7-O-glucoside, kaempferol-3,7-O-dirhamnoside (Kaempferitrin), kaempferol-3-0-α-L-rhamnoside, kaempferol-3-O-rutinoside (Nicotiflorin), kaempferol-3-arabinopyranoside, kaempferol-3-O-arabinoside (Juglanin), kaempferol (3,5,7,40 -Tetrahydroxyflavone), kynurenic acid, luteolin-7-O-rutinoside, malic acid, naringenin-7-O-glucoside (Prunin), orientin(luteolin-8-C-glucoside , pallidol, pinobanksin, pratensein, phloretin, quercetin-3-O-galactoside (Hyperin), quercetin, quercitrin (quercetin-3-0-rhamnoside), quinic acid, resveratrol, vanillic acid, vitexin-7-O-(6"-pcoumaroyl) glucoside, 3,5-dihydroxy-4,6,7-trimethoxyflavone, 5-hydroxy-6, 7, 8, 30 ,40 – pentamethoxyflavone were studied on the docking behaviour of tyrosinase by using the patchdock method.

MATERIALS AND METHODS

Ligand preparation

Chemical structures of thirty-six ligands (*Cissus rotundifolia*) namely 1) aureusidin (CID no:5281220); 2) avicularin (CID no:5490064); 3) calycosin (CID no: 5280448); 4) catechingallate (CID no: 6419835); 5) delphinidin (CID no: 68245); 6) diosmetin (CID no:5281612); 7) epiafzelechin (CID no:443639); 8) epicatechin (CID no:46231953); 9) epigallocatechin (CID no:72277); 10) gallocatechin (CID no:5276890); ,11) isohemiphloin (CID no: 42607891); 12) isoorientin-7-*O*- glucoside (CID no: 72193669); 13) kaempferol-3,7-*O*-dirhamnoside (CID no: 133564710); 14) kaempferol-3-*O*-α-L-rhamnoside (CID no: 5316673); 15) kaempferol-3-*O*-rutinoside (CID no: 5318767); 16) kaempferol-3-arabinopyranoside (CID no: 21310440); 17) kaempferol-3-*O*-arabinoside (CID no: 5318717); 18) kaempferol (CID no: 131752667); 19) kynurenic acid (CID no: 3845); 20) luteolin-7-*O*-rutinoside (CID no: 14032966); 21) malic acid (CID no : 525); 22) naringenin-7-*O*-glucoside (CID no: 92794); 23) orientin (CID

no : 5281675); 24) pallidol (CID no : 484757); 25) pinobanksin (CID no:73202); 26) pratensein (CID no: 5281803); 27) phloretin (CID no : 4788); 28) quercetin-3-*O*-galactoside (CID no: 5281643); 29) quercetin (CID no : 5280343); 30) quercitrin (CID no : 5280459); 31) quinic acid (CID no : 6508); 32) resveratrol (CID no : 445154); 33) vanillic acid (CID no : 8468); 34) vitexin-7-*O*-(6"-pcoumaroyl) glucoside (CID no : 44257759); 35) 3,5-dihydroxy-4,6,7-trimethoxyflavone (CID no : 70383) and 36) 5-Hydroxy-6, 7, 8, 30, 40 - pentamethoxyflavone (CID no : 10200272) were downloaded from PubMed database. These selected ligands were prepared according to the earlier report (7). These prepared structures were used for further study (patchdock).

Identification and Preparation of Target protein

The three-dimensional (3D) structure of the tyrosinase (PDB ID: 2Y9W with a resolution of 2.30 A°) was obtained from Protein Data Bank (PDB). A chain of tyrosinase protein was processed individually by removing other chains, ligands, and crystallographically observed water (H_2O) molecules (i.e., water without hydrogen bonds) by using UCSF Chimera software.

Docking Study

A docking study was performed for thirty six selected constituents of *Cissus rotundifolia* using the patchdock online server. Finally, the binding site analysis was carried out for the best-docked pose using PyMOL software (7).

RESULTS

Figure 1 shows the *Cissus rotundifolia* plant photo taken from Kanjanur village, Villupuram District, Tamilnadu, India, on April, 2021.

Figure 1 represents the *Cissus rotundifolia* leaf and flower.

Table 1 shows the chemical nature of thirty six selected constituents of *Cissus rotundifolia*. Out of thirty six ligands, twelve ligands belong to the flavonoid (33.3%) and flavonoid glycoside (33.3%) classes, respectively. Similarly, polyphenol (13.89%) and phenol (5.5%) are two other major classes of selected ligands.

Table 1: Nature of chemical class of thirty six selected ligands from *Cissus rotundifolia*

S.no	Ligand Name	Nature of Chemical Class
1	Aureusidin	Flavonoid
2	Avicularin	Flavonoid
3	Calycosin	Flavonoid
4	Catechin gallate	Polyphenol

5	Delphinidin	Anthocyanidin
6	Diosmetin	Flavonoid
7	3, 5-Dihydroxy-4, 6, 7-trimethoxyflavone	Flavonoid
8	Epiafzelechin	Flavonoid
9	Epicatechin	Polyphenol
10	Epigallocatechin	Polyphenol
11	Gallocatechin	Polyphenol
12	5-Hydroxy-6, 7, 8, 30, 40 – pentamethoxyflavone	Flavonoid
13	Isohemiphloin	Flavonoid
14	Isoorientin-7-*O*-glucoside	Flavonoid glycoside
15	Kaempferol-3,7-*O*-dirhamnoside	Flavonoid glycoside
16	Kaempferol-3-*O*-α-L-rhamnoside	Flavonoid glycoside
17	Kaempferol-3-*O*-rutinoside	Flavonoid glycoside
18	Kaempferol-3-arabinopyranoside	Flavonoid glycoside
19	Kaempferol-3-*O*-arabinoside	Flavonoid glycoside
20	Kaempferol	Flavonoid
21	Kynurenic acid	Quinoline monocarboxylic acid
22	Luteolin-7-*O*-rutinoside	Flavonoid glycoside
23	Malic acid	Alpha hydroxyl acid
24	Naringenin-7-*O*-glucoside	Flavonoid glycoside
25	Orientin	Flavonoid glycoside
26	Pallidol	Polyphenol
27	Pinobanksin	Flavonoid
28	Pratensein	Flavonoid
29	Phloretin	Phenol
30	Quercetin-3-*O*-galactoside	Flavonoid glycoside
31	Quercetin	Flavonoid
32	Quercitrin	Flavonoid glycoside
33	Quinic acid	Cyclic polyol
34	Resveratrol	Phenol
35	Vanillic acid	Phenolic acid
36	Vitexin-7-*O*-(6”-*p*- coumaroyl) glucoside	Flavonoid glycoside

The docking studies revealed that quercetin-3-*O*-galactoside exhibited the highest (ACE) atomic contact energy (-145.70 kcal/mol) with the tyrosinase enzyme. In contrast, epiafzelechin showed the lowest binding energy (-3.45 kcal/mol) with the target enzyme tyrosinase (as shown in table 2).

Table 2: The interaction energy analysis of thirty six selected (*Cissus rotundfolia*) ligands with the tyrosinase using the patchdock method

S.no	**Ligand**	**-ACE**	**Interactions of amino acids residues**	**Bond distance (A)**
1	Aureusidin	+1.51	Lys 379	3.3
2	Avicularin	41.01	Lys 50 Glu 67 Lys 70 Gln 74 Tyr 78	1.9 2.3 3.1 2.7 2.3

			Glu 340	2.8
3	Calycosin	27.72	Gln 74	3.4
4	Catechin gallate	+9.09	Asp 312	2.9 and 3.5
5	Delphinidin	96.74	Ala 246 Glu 322	2.1 2.7
6	Diosmetin	18.69	Asp 312 Ser 364	3.4 3.4
7	3, 5-Dihydroxy-4, 6, 7-trimethoxyflavone	43.24	Thr 308	3.4
8	Epiafzelechin	3.45	Thr 308 Asp 312 Glu 359 Lys 379	3.1 2.2 2.4 3.1
9	Epicatechin	144.25	Lys 70 Gln 74	3.4 2.7
10	Epigallocatechin	26.60	Asp 312 Trp 358	2.9 2.6
11	Gallocatechin	19.15	Gln 307 Asp 312	2.5 and 2.2 3.6
12	5-Hydroxy-6, 7, 8, 30, 40 – pentamethoxyflavone	+16.88	Asp 312	2.8
13	Isohemiphloin	30.58	Thr 303 Gln 307 Asp 312 Asp 357 Lys 379	3.3 3.3 3.1 2.6 2.9
14	Isoorientin-7-*O*-glucoside	143.31	Ala 246 Asn 260 Ala 323	2.8 2.5 1.7
15	Kaempferol-3,7-*O*-dirhamnoside	67.14	Gln 307	3.3 and 3.1
16	Kaempferol-3-*O*-α-L-rhamnoside	100.67	Gln 307 Asn 312 Glu 356	2.8 3.3 2.6
17	Kaempferol-3-*O*-rutinoside	23.04	Lys 70 Gln 72 Gln 74	1.9 2.4 3.1 and 3.0
18	Kaempferol-3-arabinopyranoside	12.52	Lys 376 Gln 307 Glu 359 Lys 374 Lys 379	3.4 2.4 2.3 2.3 2.7 and 3.0 and 2.0
19	Kaempferol-3-*O*-arabinoside	4.73	Gln 307 Lys 379	3.4 2.0 and 2.6
20	Kaempferol	121.8	Gln 159	3.4
21	Kynurenic acid	38.87	Gln 307 Thr 308	3.2 3.5
22	Luteolin-7-*O*-rutinoside	39.35	Lys 4 Glu 67	2.5 2.6

			Lys 70 Pro 73 Gln 74 Tyr 82 Tyr 343	3.2 and 2.3 2.5 2.5 3.4 3.0
23	Malic acid	16.80	Met 319 Arg 321	2.6 3.2
24	Naringenin-7-*O*-glucoside	57.58	Gln 74	2.6
25	Orientin	6.33	Gln 307 Lys 379	3.1 and 2.5 2.9
26	Pallidol	107.00	Thr 308 Ser 364	2.8 2.7
27	Pinobanksin	+1.06	Asn 19 Ser 364	2.6 3.4
28	Pratensein	33.83	Asp 312 Asp 357	2.9 3.1
29	Phloretin	37.8	Thr 308	3.2
30	Quercetin-3-*O*-galactoside	145.70	Gln 44 His 178 Lys 180 Glu 196	3.5 3.3 3.3 3.5
31	Quercetin	14.29	Lys 379	2.1
32	Quercitrin	+16.28	Gln 307 Asp 312 Glu 356	3.0 2.9 2.0
33	Quinic acid	+13.21	Ile 17 Asn 19 Ser 364	2.2 3.3 3.4
34	Resveratrol	39.33	Lys 70 Tyr 343	2.6 3.0
35	Vanillic acid	44.65	Asp 357	2.7
36	Vitexin-7-*O*-(6”-*p*- coumaroyl) glucoside	+16.4	Thr 308	2.5 and 3.0

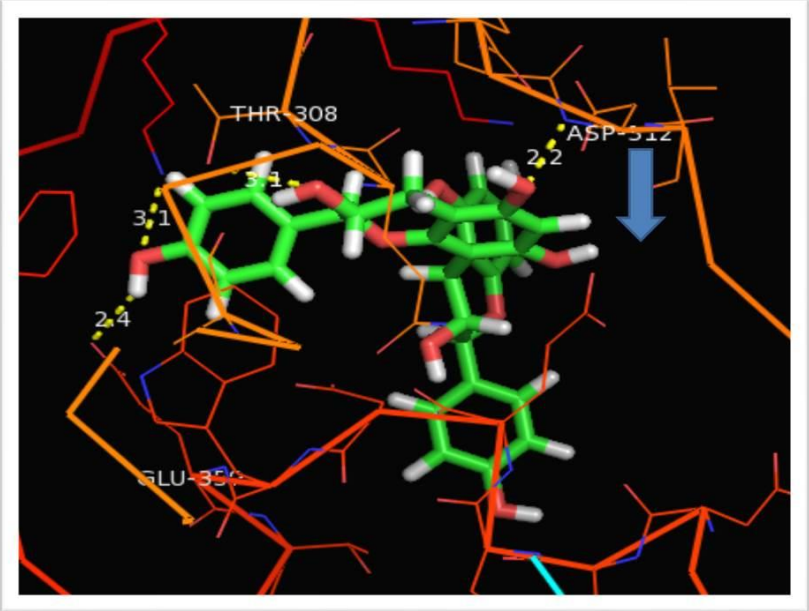
The binding energy results showed the following order: quercetin-3-*O*-galactoside (--145.70 kcal/mol), < epicatechin (-144.25 kcal/mol), < isoorientin-7-*O*-glucoside (-143.31 kcal/mol), < kaempferol (-121.8 kcal/mol), < pallidol (-107.00 kcal/mol), < kaempferol-3-*O*-α-L-rhamnoside (-100.67 kcal/mol), < delphinidin (-96.74 kcal/mol), < kaempferol-3,7-*O*-dirhamnoside (-67.14 kcal/mol), < naringenin-7-*O*-glucoside (-57.58 kcal/mol), < vanillic acid (-44.65 kcal/mol), < 3, 5-Dihydroxy-4, 6, 7-trimethoxyflavone (-43.24 kcal/mol), < avicularin (-41.01 kcal/mol), < resveratrol (-39.33 kcal/mol), < luteolin-7-*O*-rutinoside (-39.35 kcal/mol), < kynurenic acid (-38.87 kcal/mol), < phloretin (-37.80 kcal/mol), < pratensein (-33.83 kcal/mol), < isohemiphloin (-30.58 kcal/mol), < calycosin (-27.72 kcal/mol), < epigallocatechin (-26.60 kcal/mol), < kaempferol-3-*O*-rutinoside (-23.04 kcal/mol), < gallocatechin (-19.15 kcal/mol), < diosmetin (-18.69 kcal/mol), < quercetin (-14.29 kcal/mol), < kaempferol-3-arabinopyranoside (-12.52 kcal/mol), < orientin (-6.33 kcal/mol), < kaempferol-3-*O*-arabinoside (-4.73 kcal/mol), < epiafzelechin (-3.45 kcal/mol).

In the present study, seven ligands namely aureusidin (+1.51 kcal/mol); catechin gallate (+9.09 kcal/mol); 5-Hydroxy-6, 7, 8, 30, 40 – pentamethoxyflavone (+16.88 kcal/mol); pinobanksin (+1.06 kcal/mol); quercitrin (+16.28 kcal/mol), quinic acid (+13.21 kcal/mol)

and vitexin-7-*O*-(6"-*p*- coumaroyl) glucoside (+16.4 kcal/mol) have shown poor (ACE) atomic contact energy values. More the negative atomic contact energy (ACE) value of ligands indicates a good binding affinity towards the target enzyme. In contrast more, the positive atomic contact energy (ACE) value of ligands indicates a poor binding affinity towards the target enzyme (tyrosinase).

Ten ligands such as catechin gallate, diosmetin, epiafzelechin, epigallocatechin, gallocatechin, 5-hydroxy-6, 7, 8, 30, 40 – pentamethoxyflavone, isohemiphloin, kaempferol-3-*O*-α-L-rhamnoside, pratensein, and quercitrin were shown to have an interaction with the Asp 312 amino acid residue of the tyrosinase enzyme (Fig. 2).

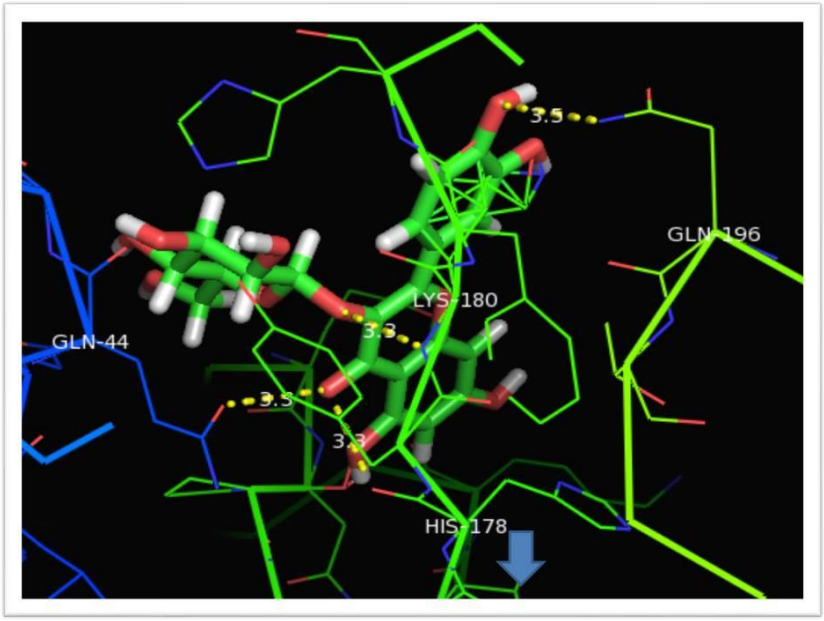
Figure 2 represents an interaction of epiafzelechin with the target enzyme tyrosinase



Note: ↓ Down arrow mark represents the Aspartic acid (Asp) amino acid at position 312 of the tyrosinase enzyme interact with the ligand (epiafzelechin). Dotted lines represent the hydrogen bonds.

Similarly nine ligands, namely gallocatechin, isohemiphloin, kaempferol-3,7-*O*-dirhamnoside, kaempferol-3-*O*-α-L-rhamnoside, kaempferol-3-arabinopyranoside, kaempferol-3-*O*-arabinoside, kynurenic acid, orientin, and quercitrin were shown to have an interaction with the Gln 307 amino acid residue of the tyrosinase enzyme. Furthermore, seven ligands include aureusidin, epiaflechin, isohemiphloin, kaempferol-3-arabinopyranoside, kaempferol-3-*O*-arabinoside, orientin, and quercetin were shown to have an interaction with the Lys 379 amino acid residue of the tyrosinase enzyme. Interestingly one ligand (quercetin-3-*O*-galactoside) was shown to have an interaction with the His 178 amino acid residue of the tyrosinase enzyme (Fig. 3)

Figure 3 represents an interaction of quercetin-3-*O*-galactoside with the target enzyme tyrosinase.



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Note: ↓ Down arrow mark represents the Histidine (His) amino acid at position 178 of the tyrosinase enzyme interact with the ligand (quercetin-3-*O*-galactoside). Dotted lines represent the hydrogen bonds.

DISCUSSION

The traditional medicine uses, phytochemistry, biological activities, and therapeutic uses of *Cissus* species and their chemical or bioactive constituents are of appreciable to researchers (11-17; 7). Moreover the paleoecology study provides new evidence for the presence of *Cissus rusingensis* on Rusinga Island during the early Miocene period (18). *Cissus glauca* has been used as natural admixture in ancient temples and monuments in Kerala (India) which increases the durability of hydraulic lime mortars used in repair purpose (19).

Tyrosinase is a copper-containing enzyme which catalyzes the key regulatory steps in melanin biosynthesis pathway. Increased melanin biosynthesis which results in excessive accumulation of melanin that give rise to dermatological problems (like melanoma, melasma, and lentigo simplex, etc) and idiopatic diseases (20-21). Thus tyrosinase inhibitors have both cosmetic (22) and medicinal applications (23).

The background mentioned above, prompts us to carry out the present study with thirty six selected constituents of *Cissus rotundifolia*, where majority of them belong to both flavonoid and flavonoid glycoside class (24). In the previous study, we had reported that 11 constituents of *Cissus rotundifolia*, namely aconitic acid, astragalin, acteoside, alliospiroside A, beta amyrin, bergenin, formononetin, gallic acid, isovitexin, isoorientin, and isoquercitrin as tyrosinase inhibitors using the patchdock method (7).

Kaempferol-3-*O*-rutinoside, kaempferol, quercetin, resveratrol, and vanillic acid have been reported to possess tyrosinase inhibition (24-28) these results are on par with the present result. In the present study, ten ligands such as catechin gallate, diosmetin, epiafzelechin, epigallocatechin, gallocatechin, 5-hydroxy-6, 7, 8, 30, 40 – pentamethoxyflavone, isohemiphloin, kaempferol-3-*O*-α-L-rhamnoside, pratensein, and quercitrin were shown to have an interaction with the Asp 312 amino acid residue of the tyrosinase. This result has shown good agreement with the previous report (29). Similarly nine ligands, namely gallocatechin, isohemiphloin, kaempferol-3,7-*O*-dirhamnoside, kaempferol-3-*O*-α-L-rhamnoside, kaempferol-3-arabinopyranoside, kaempferol-3-*O*-arabinoside, kynurenic acid, orientin, and quercitrin were shown to have an interaction with the Gln 307 amino acid residue of the tyrosinase. This result has shown good agreement with the previous report (29). Moreover, seven ligands include aureusidin, epiafzelechin, isohemiphloin, kaempferol-3-

arabinopyranoside, kaempferol-3-*O*-arabinoside, orientin, and quercetin were shown to have an interaction with the Lys 379 amino acid residue of the tyrosinase. This result has shown good alignment with the previous report (29). Furthermore, one ligand (quercetin-3-*O*-galactoside) has shown to have an interaction with the His 178 amino acid residue of the tyrosinase. This result has shown good agreement with the previous report (8).

CONCLUSION

In the present study, all the thirty six selected constituents of *Cissus rotundifolia* have been shown to dock with the target enzyme tyrosinase. Interestingly one ligand (quercetin-3-*O*-galactoside) has shown to have an interaction with the His 178 amino acid residue of the tyrosinase. These results provide new insight in understanding their inhibitory activities and pave the way for further *in vitro* investigation of these 36 selected *Cissus rotundifolia* constituents as possible inhibitors against the enzyme tyrosinase.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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