International Journal of Phytocosmetics and Natural Ingredients 2015;2:6

doi 10.15171/ijpni.2015.06

Original Article

Piper Genus: Source of Natural Products With Anti-tyrosinase Activity Favored in Phytocosmetics

Fabiana Almeda, Laura Astorga, Andrea Orellana, Ligia Sampuel, Paola Sierra, Isabel Gaitán, Armando Cáceres*

Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala, Guatemala

Correspondence to
Armando Cáceres, Departmento de Citohistología,
Facultad de Ciencias Químicas y Farmacia,
Universidad de San Carlos, Guatemala
Email:
acaceres46@gmail.com

Received 28 February 2015
Accepted 7 May2015
ePublished 15 June 2015

Abstract

Tyrosinase is an enzyme part of the melanogenic cycle, which provides color to skin, hair and eyes; when present in high concentrations it produces hyperpigmentation. The aim of this study was to determine tyrosinase inhibitory activity of extracts (dichloromethane and methanol) from ten Guatemalan species of the genus *Piper* (*P. amalago, P. auritum, P. hispidum, P. jacquemontianum, P. oradendron, P. psilorhachis, P. retalhuleuense, P. sempervirens, P. umbellatum and P. variabile*). The inhibitory effect of tyrosinase activity was determined by enzymatic assays, qualitative thin layer chromatography (TLC) and quantitative by spetrophotometric kinetic method. Samples were analyzed in quintuplicate and reference standard solutions of kojic acid were used to verify the effectiveness of the tests, being all statistically significant at a confidence interval of 95%. All the extracts demonstrated some degree of inhibition by the bioautographic procedure, in four extracts activity has been confirmed by quantitative spectrophotometry. Extracts with the best inhibition activity in both solvents were *P. variabile* (IC50 2.0±0.1 µg/mL) and P. umbellatum (IC50 4.9±0.2 µg/mL); and, methanol extract of *P. jacquemontianum* (IC50 6.8±0.3 µg/mL) and *P. psilorhachis* (IC50 6.5±0.3 µg/mL). Kojic acid activity was higher than Piper extracts, but the analyzed extracts deserve further evaluation.

Keywords: Skin whitening, *Piper variabile, Piper umbellatum,* Hyperpigmentation

Introduction

Hyperpigmentation, such as senile lentigo, melasma, pigmented freckles and acne scars are of interest to dermatologists and cosmetologist, but melanin pigments are also found in the brain of mammals, by tyrosinase enzyme that plays a role in neuromelanine forming in the brain.[1] This mixed function could be critical for dopamine neurotoxicity and can contribute to the neurodegeneration associated with Parkinson's disease.[2]

Tyrosinase is a key enzyme in melanin biosynthesis in plants and animals. Tyrosinase inhibitors may be clinically useful for the treatment of certain dermatological conditions associated with hyperpigmentation high melanin production; they also find applications in cosmetics for depigmentation after burns.[3] Additionally, tyrosinase is involved in the process of insect molting and adhesion of marine organisms. Tyrosinase inhibitors have been used in cosmetic products that promote skin lightening.[4]

Melanogenesis is a physiological process that results in the synthesis of melanin pigments, which play a crucial role in the protection of skin photocarcinogenesis. The key component of normal skin color is provided by the melanin, tyrosinase is a key enzyme in the biosynthesis of melanin, it mainly focuses on catalyzing 2 reactions in the synthesis process. Hydroxylation of L-tyrosine (tyrosine monophenolic activity) to 3,4-dihydroxy-phenylalanine (L-DOPA) and the oxidation of L-DOPA (diphenolase activity), to give dopaquinone.[5,6] The use of tyrosinase inhibitors such as kojic acid and hydroquinone is increasingly important in the cosmetic industry due to its anti-pigmentation effects.[7] Recent findings show that 4-*n*-butylresorcinol had the potential to acto as an effective depigmentation agent high better security profile than currently available agents.[8]

The search for tyrosinase inhibition activity in natural products has demonstrated that vegetal extracts could be a potential source of new ingredients or isolated molecules. In a screening of tropical plants from Brazil, 67 species from 38 familias were studied, demonstrating good activity in 5 (7.46%) species (*Stryphnodendron barbatimao, Portulacca pilosa, Cariniana basiliensis, Entada africana* and *Prosopis africana*).[9] From 91 native plants from Central Argentina, 18 (19.78%) showed inhibitory activity higher than 90%; *Dalea elegans, Lepechinia meyenii* and *Lithrea molleoides* were the most potent (IC_{50} 0.48, 10,43 and 3.77 µg/ml respectively); (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol isolated from *L. molleoides*, was 37 times more active than kojic acid.[4]

The family *Piperaceae,* is a basal angiosperm present on the surface of the earth for millions of years. The 2 largest

Please cite this paper as: Almeda F, Astorga L, Orellana A, et al. Piper genus: source of natural products with anti-tyrosinase activity favored in phytocosmetics. *International Journal of Phytocosmetics and Natural Ingredients*. 2015;2:6. doi:10.15171/ijpni.2015.06.

genuses are *Piper* and *Peperomia*, each containing about 1000 species.[10,11] They are represented by herbs, shrubs and trees, widely distributed in the tropical and subtropical regions of the world, several species are used by men as condiment, food, ornamentals, perfumes, oils, fish bait, fish poison, insecticides, hallucinogens, and many medicinal uses since ancient times.[13-18] Besides, the interaction with disperser animals such as bats or with caterpillars and beetles has indicated *Piper* as an important model species to investigate tropical ecology.[11] The biogeography of the genus indicates a great diversity in the American continent, where 3 biogeographic provinces (Central America, Mexico and Colombian northwest, the Amazon basin and the Atlantic forest of Brazil) are described.[12]

Piper species have high commercial, economical and medicinal importance; particularly for pepper (*Piper nigrum*) in the worldwide spice markets and for its wide range of biological activities,[19] several chemical and biological investigations are known for their medicinal interest, mainly essential oils.[20]

Materials and Methods

Plants and Extracts

Samples from 10 species of genus *Piper* were collected from cropland or management fields in Alta Verapaz or Suchitepequez, according to good collection practices, including permission to collect, technical planning, selection of representative flowering specimens, identification by a biologist (Luis Alvarez) from Farmaya Laboratory Herbarium (CFEH), confirmation by a botanist (Mario Veliz, BIGU Herbarium, USAC), and deposit of voucher specimens at CFEH Herbarium from Farmaya Laboratories (Table 1).

Grounded shade-dried vegetal (500 g, about mesh No. 60) materials were extracted by sequential percolation with dichloromethane for 2 days followed by extraction with methanol for 3 days; both eluents were concentrated by rotary evaporator, and dried in a vacuum dryer.

Tyrosinase Inhibitory Activity

Qualitative activity was determined by thin layer chromatography (TLC).[7] A solution of L-tyrosinase (3,333 U/ml) was prepared by dissolving 2 mg in 10 mL of phosphate buffer (PBS, pH 6.5, 50 mM). The substrate was prepared by dissolving 3.6 mg of L-tyrosine solution in 10 ml of PBS. The methanol extract was prepared by dissolving 20 mg in 50 mL of DMSO and 950 ml of acetone, the dichloromethane extract was prepared by dissolving 20 mg in 1 mL of acetone. The positive control contained 1 mg of kojic acid in 10 ml of demineralized water. The extracts (10 µL) dissolved in dichloromethane and/or methanol were added on silica gel plates, allowed to dry, and placed in the chromatographic chamber previously saturated with acetone:methanol (1:49). Plates were remove from the mobile phase and allowed to dry. Plate with L-tyrosine as a substrate (2 mM) was sprayed and incubated at 25°C for 10 minutes; plate with L-tyrosinase solution was sprayed and incubated at 25°C for 30 minutes. Gray-purple and clear zones of inhibition of the enzyme L-tyrosinase coloration was observed, indicating the active compounds in the extracts.

Quantitative antityrosinase activity was determined spectrophotometrically.[4,7] L-tyrosinase (0.20 mg) was dissolved in 10 mL of phosphate buffer (pH 6.5, 50 mM), 20 mg of extracts and purified compounds was dissolved in DMSO to give concentrations (25 to 400 mg/mL), and this stock solution was diluted to 600 µg/mL in the phosphate buffer. Kojic acid was used as a positive control (20 mg/mL); 70 mL of extracts and standard sample were combined with 30 mL of tyrosinase in triplicate in a 96-well microtiter plate, and was incubated at 37°C for 60 minutes. The reaction mixture to 110 µl of substrate (2 mM L-tyrosine) was added and incubated at 37°C for 15 minutes. The change in absorbance at 492 nm was measured.[4,7]

Results

Extracts with 2 organic solvents were obtained by sequential extraction, showing yields from, 6.97% to 15.53% for dichloromethane, and 3.29% to 8.63% for methanol (Table 1).

In Table 2 it can be observe that the 20 extracts analyzed by TLC showed some degree of tyrosinase inhibitory activity. Some had a double inhibition band, indicating that at least 2 molecules might be implicated in this activity. It

Table 1. Procedence and Extraction Yield of 10 *Piper* Species From 2 Sites of Guatemala

Piper Species (Leaves)	Place of Collection	CFEH Voucher	Extraction Yield (%)	
			Dichlomethane	Methanol
P. amalago L.	Samayac, Such.	1073	7.96	3.52
P. auritum L.	Samayac, Such.		6.26	7.54
P. hispidum Swartz	Samayac, Such.	1071	9.18	7.98
P. jacquemontianum Kunth.	Samayac, Such.	1069	7.37	8.63
P. oradendron Trel. & Standl.	Samayac, Such.	1075	8.41	7.34
P. psilorhachis C. DC	Chisec, AV	1143	10.71	5.42
P. retalhuleuense Trel. & Standl.	Sto. Domingo, Such.	1053	6.97	7.25
P. sempervirens (Trel.) Lundell	Chisec, AV	1113	11.49	6.33
P. umbellatum L.	Samayac, Such.	1070	8.89	7.43
P. variabile C. DC ex Donn.-Sm.	Chisec, AV	1140	15.53	3.29

Table 2. Qualitative and Quantitative Determination of Tyrosinase Inhibition Activity of Extracts From *Piper* Spp From Guatemala[a]

Piper Specie	Solvent	Qualitative Determination by TLC			Quantitative Determination by Spectrophotometry		
		Rf1	Rf2	Result	IC_{50} µg/ml	95% CI	µgAKE/mg
P. amalago	Methanol	1.3	-	++[b]	10.4 ±0.8	9.6-11.2	7.96 ±0.3
	Dichloromethane	2.1	-	+[c]	34.8 ±1.6	33.2-36.8	20.1 ±0.9
P. auritum	Methanol	1.3	-	+	42.8 ±3.2	39.6-46.0	37.5 ±0.5
	Dichloromethane	1.7	-	++	15.0 ±0.8	14.2-15.8	58.4 ±1.2
P. hispidum	Methanol	1.3	-	+	45.2 ±2.8	42.4-48.0	62.3 ±2.1
	Dichloromethane	1.6	-	+	6.8 ± 0.3	45.1-48.5	74.5 ±0.4
P. jacquemontianum	Methanol	1.4	-	++	28.5 ±0.8	6.5-7.1	16.0 ±0.7
	Dichlorometane	2.1	-	+	30.8 ±1.2	27.7-29.3	65.8 ±1.3
P. oradendron	Methanol	1.9	-	+	38.7 ±1.9	29.6-32.0	36.4 ±0.2
	Dichlorometane	1.6	-	+	6.5 ± 0.3	36.8-40.6	71.1 ±2.6
P. psilorhachis	Methanol	1.0	1.7	++	19.8 ±0.8	6.2-6.8	15.9 ±0.9
	Dichlorometane	2.6	-	++	12.5 ±0.4	19.0-20.6	64.5 ±3.1
P. retalhuleuense	Methanol	1.4	-	++	7.6 ± 0.5	12.1-12.9	38.5 ±2.1
	Dichlorometane	2.3	2.9	++	19.0 ±0.7	7.1-8.1	44.2 ±1.4
P. sempervirens	Methanol	2.0	-	++	7.9 ± 0.3	18.3-19.7	36.5 ±1.2
	Dichlorometane	3.4	4	++	6.6 ± 0.2	7.6-8.2	48.9 ±2.4
P. umbellatum	Methanol	1.3	-	++	4.9 ± 0.2	6.4-6.8	31.3 ±1.3
	Dichlorometane	5.0	-	++	2.1 ± 0.1	4.7-5.1	20.0 ±0.8
P. variabile	Methanol	0.9	1.7	++	2.0 ± 0.1	2.0-2.2	5.4 ± 0.2
	Dichlorometane	6.1	-	++	1.2 ±0.07	1.9-2.1|	4.4 ± 0.2
Kojic acid	Standard	5.2	-	+++[d]		1.1-1.3	NA

Abbreviations: Rf1: Retention factor of the first compound; Rf2: Retention factor of the second compound. AKE: Kojic acid equivalents/mg of extract; NA: Not applicable.

[a] *P* = 0.032 (Binomial test), n =5 repititions; [b] (++): Moderate inhibition; [c] (+): slight inhibition; [d] (+++) High inhibition.

can also be observed the quantitative inhibition concentration 50% (IC_{50}), the confidence interval of 95%, and the kojic acid equivalent in mg per g; values of kojic acid standard are included.

The extracts that showed better tyrosinase inhibitory activity (IC_{50} 1.2-2.0 µg/ml) were *P. variabile* and *P. umbellatum*; kojic acid gave an IC_{50} of 1.2 ± 0.1 µg/ml**.** The extract with a better inhibition in both solvents was *P. variabile* presenting similar IC_{50}.

Discussion

This paper focused on studying ten species of genus *Piper*. Many of these plants are known as "cordoncillo" and are credited with analgesic, antirheumatic, diuretic, stimulant, digestive, antiulcer, antihelmintic, antibacterial and aromatic properties, and are widely used for the treatment of vaginitis, bowel disorders and as an antimicrobial.[21] Due to this wide variety of uses, 10 species were selected for screening of the tyrosinase inhibitory activity.

The extractive yield obtained in the methanol extract was better than in the dichloromethane except for *P. auritum, P. jacquemontianum* y *P. retalhuleuense.* The best yields with methanol were *P. variabile* (15.53%), *P. sempervirens* (11.49%) and *P. psilorhachis* (10.71%); in the dichloromethane extracts, the best yield was obtained from *P. jacquemontianum* (8.63%).

The variation of the yield percentages are affected by factors such as the extraction process, the nature of the (polar or non-polar) metabolites, the solvent used, the time of the year that it is collected and the place of origin or cultivation.[21]

To validate the methodology, dose-effect curves were prepared with kojic acid as standard and adapted to standard laboratory conditions.[7] Qualitative and quantitative methodologies were standardized to evaluate the activity for potential application in cosmetology. According to Kim et al,[22] tyrosinase is the rate-limiting enzyme in the biosynthesis of melanin pigments responsible for the color of hair, skin and eyes. Tyrosinase inhibitors have been used in cosmetic products that promote skin lightening,[4] but most of them do not meet all the requirements of clinical efficacy, and adverse effects are observed.[2] Consequently, alternative agents are substances derived from plants that have been proven safe and effective supplement for the treatment of certain disorders in humans.[23]

This study demonstrated that some species of *Piper,* have tyrosinase inhibitory activity. The presence of tyrosinase inhibitory activity in organic extracts was determined by a qualitative TLC method, revealing positive activity due to a chelating effect, since the active site of the species

separates the metal ion of the enzyme (Cu^{+}), forming a complex system that inhibit the metal ion to participate or catalyze the substrate-enzyme reaction, that will showed a clear zones of inhibition of tyrosinase in all dichloromethane extracts tested.

Four of the analyzed extracts showed 2 inhibition bands in the methanol extract, implying that at least 2 molecules might be involved in tyrosinase inhibition activity; pevious extraction with dichloromethane eliminated fats, due to its hydrophobic character, which helps eliminate false positives.[4]

The best inhibitory activity of tyrosinase inhibition activity was demonstrated by the dichloromethane (IC_{50} 2.0 µg/mL) and methanol (IC_{50} de 2.1 µg/mL) extract of *P. variabile,* because it inhibited 50% of tyrosinase with the least amount of extract as interpreted by Momtaz et al.[7]

The second best activity was the dichloromethane extract of *P. umbellatum* (IC_{50} 4.9 µg/mL), followed by the methanol extract of *P. psilorachis* (IC_{50} 6.5 µg/ml), while the one with the smallest activity was the dichloromethane extract of *P. hispidum* (IC_{50} 46.8 µg/mL).

When comparing the results of the extracts with the reference standard can be seen that the standard has a higher tyrosinase inhibitory activity than the extracts analyzed, the species closest to the CI_{50} of the standard is the dichloromethane extract of *P. variabile*. The identification of chemical compounds in this species, capable of modulating the metabolism of pigmentation is of great interest. It should be discarded a false positive caused by enzyme inhibition by the interference of fat since this is a nonpolar solvent, but this should be confirmed.[24]

The extract that showed the least amount of equivalents was *P. variabile,* in the dichloromethane extract (4.4 ± 0.2 µg EAK/mg) and methanol (5.4 ± 0.2 µg EAK/mg), followed by methanol extracts *P. amalago* (7.9 ± 0.3 µg EAK/mg), dichloromethane extract of *P. psilorhachis* (15.9 ± 0.3 µg EAK/mg), and finally methanol extract of *P. jacquemontianum* (16.0 ± 0.3 µg EAK/mg). The species that reported the greater amount of EAK were the dichloromethane extract of *P. hispidum* (71.1 ± 0.3 µg EAK/mg) and *P. sempervirens* (66.9 ± 5.4 µg EAK/mg) therefore less tyrosinase inhibitory activity is expected, since the lower the value of µg EAK/mg extract expect the greater inhibition.[3]

In the literature reviewed no information was found from previous studies about the antityrosinase activity of the *Piper* species included in this study, so it is necessary further studies to give clear evidence of its lightening or whitening power and clinical efficacy, as well as chemical and toxicological studies of the plants that inhibited more than 50% for use in human benefit.[2] With these findings evidence is presented about the potential of the genus *Piper* for use as potent skin whitening agents.

Acknowledgements

We thank Bioassays Laboratory, Department of Cytohistology, and Natural Products Research Laboratory (LIPRONAT) both in Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos, which made this research possible. Financial support was partially provided by National Council for Science and Technology (grant FODECYT 27-2011).

References

1. Tripathi RK, Hearing VJ, Urabe K, Aroca P, Spritz RA. Mutational mapping of the catalytic activities of human tyrosinase. J Biol Chem. 1992;267:23707-23712.
2. Casañola-Martin GM, Marrero-Ponce Y, Hassan MT, et al. Dragon method for finding novel tyrosinase inhibitors: Biosilico identification and experimental in vitro assays. Eur J Med Chem. 2007;42(11-12):1370-1381. doi:10.1016/j.ejmech.2007.01.026.
3. Lin YP, Hsu FL, Chen CS, Chern JW, Lee MH. Constituents from the Formosan apple reduce tyrosinase activity in human epidermal melanocytes. Phytochemistry. 2007;68(8):1189-1199. doi:10.1016/j.phytochem.2007.02.001.
4. Chiari M, Joray M, Ruiz G, Palacios S, Carpinella M. Tyrosinase inhibitory activity of native plants from central Argentina: Isolation of an active principle from Lithrea molleoides. Food Chem. 2010;120(1):10-14. doi:10.1016/j.foodchem.2009.09.061.
5. Lee K, Kim BJ, Kim JH. Biological screening of 100 plant extracts for cosmetic use (1): Inhibitory activities of tyrosinase and DOPA auto-oxidation. Int J Cosmet Sci. 1997;19:291-298. doi:10.1046/j.1467-2494.1997.171725.x.
6. Kyeong R, Kim G, Hyun C, Sang D, Ho N. Compounds with tyrosinase inhibition, elastase inhibition and DPPH radical scavenging activities from the branches of Distylium racemosum Sieb. et Zucc. Phytother Res. 2011;25:1451-1456. doi:10.1002/ptr.3439
7. Momtaz S, Mapunya BM, Houghton PJ, et al. Tyrosinase inhibition by extracts and constituents of Sideroxylon inerme L. stem bark, used in South Africa for skin lightening. J Ethnopharmacol. 2008; 119(3):507-512. doi:10.1016/j.jep.2008.06.006.
8. Shin JW, Park KC. Current clinical use of depigmentation agents. Dermatol Sinica. 2014;32:205-210
9. Baurin N, Arnoult E, Scior T, Do DT, Berbard P. Preliminary screening of some tropical plants for anti-tyrosinase activity. J Ethnopharmacol. 2002;82:155-158.
10. Standley P, Williams L. Flora of Guatemala. Fieldiana: Botany 1976;24(1):331-332.
11. Dyer LE, Palmer ADN. Piper. A Model Genus for Studies of Phytochemistry, Ecology and Evolution. New York: Kluwer Academic/Plenum Publishers; 2004.
12. Marquis RJ. Biogeography of neotropical Piper. In: Dyer LE, ADN Palmer, eds. Piper. A Model Genus for Studies of Phytochemistry, Ecology and Evolution. New York: Kluwer Academic/Plenum Publishers; 2004:78-96.

13. Cronquist H. An Integrated System of Classification of Flowering Plants. New York: Columbia University Press; 1981.
14. Parmar VS, Jain SC, et al. Phytochemistry of the genus Piper. Phytochemistry. 1997;46:597-673.
15. Takhtajan AL. Diversity and Classification of Flowering Plants. New York: Columbia University Press; 1997.
16. Barrett B. Medicinal plants of Nicaragua's Atlantic Coast. Econ Bot. 1994;48:8-20. doi:10.1007/bf02901375.
17. Joly LG. Feeding and trapping fish with Piper auritum. Econ Bot. 1981;35:383-390.
18. Schultes RE, Raffauf RF. The Healing Forest. Portland, Oregon: Timber Press, 1990. doi:10.1007/BF02858588.
19. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Ali Khan M. Biological role of Piper nigrum L. (black pepper): A review. Asian Pacific J Trop Biomed. 2012;2(3):S1945-S1953. doi:10.1016/S2221-1691(12)60524-3
20. Mundina M, Vila R, Tomi F, et al. Leaf essential oils of three Panamanian Piper species. Phytochemistry. 1998;47:1277-1282.
21. Gómez A. Caracterización de extractos y aceites esenciales y evaluación de la actividad biológica de hoja de tres especies Piperaceas (P. jacquemontianum, P. oradendron y P. umbellatum) [Bachelor thesis]. Guatemala: Universidad de San Carlos de Guatemala; 2008.
22. Kim YM, Yun J, Lee C, Lee H, Min K, Kim Y. Oxyresveratrol and hidroxystilbene compounds, inhibitory effect on tyrosinase and mechanism of action. J Biol Chem. 2002;277(18):16340-16344. doi:10.1074/jbc.m200678200.
23. Cai X, Lin M, Shan S, Qi X, Ying Z. Curcumin inhibits melanogenesis in human melanocytes. J Ethnopharmacol. 2012;26:174-179. doi:10.1002/ptr.3517.
24. Curto E, Kwong C, Hermersdorfer H, et al. Inhibitors of mammalian melanocyte tyrosinase: in vitro comparisons of alkyl esters of gentisic acid with other putative inhibitors. Biochem Pharmacol. 1999;57:663-672.