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Leaves of *Duroia longiflora*: Isolation of a Biflavanoid and Histochemical Analysis

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Abstract

Duroia longiflora (Rubiaceae) is an Amazonian plant species with no previous chemical study. The aim of this work was to study the chemical composition of *D. longiflora* leaves and study their histochemistry. *In vivo* tissues of the leaves were histolocalized. Extracts were made from dried leaves with hexane, methanol, and water, and the methanolic extract was fractionated. Fractionation of the dichloromethane phase of the methanolic leaf extract allowed the isolation of a biflavanoid. Via nuclear magnetic resonance (mono and bidimensional), mass spectrometer analysis, and comparison with existing literature, its chemical structure was identified as 2α,3α-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4β→8)-epicatechin. This is the first report describing the histochemical and phytochemical studies of *D. longiflora* leaves. As far as we know, this is the first report of this substance in any Rubiaceae species.

Keywords

phytochemistry, leaf anatomy, histochemistry, Rubiaceae

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The Amazon rainforest is considered to be one of the greatest sources of biodiversity on the Planet.[1] One of the largest flowering plant families in the tropics is Rubiaceae represented, in Brazil, by 126 genera and 1397 species,[2] and it is characterized by the production of a wide variety of secondary metabolites including terpenes, flavonoids, alkaloids, and coumarins.[3]

Duroia longiflora Ducke (Rubiaceae) belongs to the subfamily Ixoroideae, tribe Gardenieae, and are woody plants, with whole stipules, corollas with contour lobes, rarely one fruit fleshy, individuals can reach 17 meters in height and 15 cm in diameter,[4] the inner bark (rhytidome) is dark brown, striated, and fissured, and the outer bark is brown with cream striations.[5] Literature search revealed only few chemical studies with *Duroia* species: two were conducted with *Duroia hirsuta* describing flavonoids and iridoid isolation[6,7] and some with *Duroia macrophylla* reporting the isolation of indole alkaloids with antitumor and antituberculosis activity, along with two triterpenes with antituberculosis activity as well.[8-14] So, we decided to investigate other species of the genus *Duroia*, *D. longiflora*.

Histochemistry coupled with phytochemistry can provide an excellent chemical profile. Histochemical studies detected the presence of flavonoids and flavones when stained with ferric chloride in the leaf tissue, and when stained with hydrochloric vanillin, a specific reagent for tannins, it revealed an abundance of tannins along the tissue (Figure 1a and b).

Our observations showed tannins are restricted to epidermis and wall of collenchyma, phloem, and trichomes. This information guided the phytochemical fractionation on the search of flavonoids and tannins, due to the abundance seen in the histochemical test. Phytochemical fractionation led to the isolation of a biflavanoid, identified as 2α,3α-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4β→8)-epicatechin (Figure 2 and Supplementary Material). This is the first report of this substance in any Rubiaceae species, as far as we know. Only two studies reporting the presence of this substance were found in literature, both in Sapindaceae species: in the seeds of *Litchi chinensis* [15] and in the husks of *Xanthoceras sorbifolium*.[16]

Duroia species suffers with herbivory and the species *D. hirsuta* and *Duroia saccifera* developed myrmecophily

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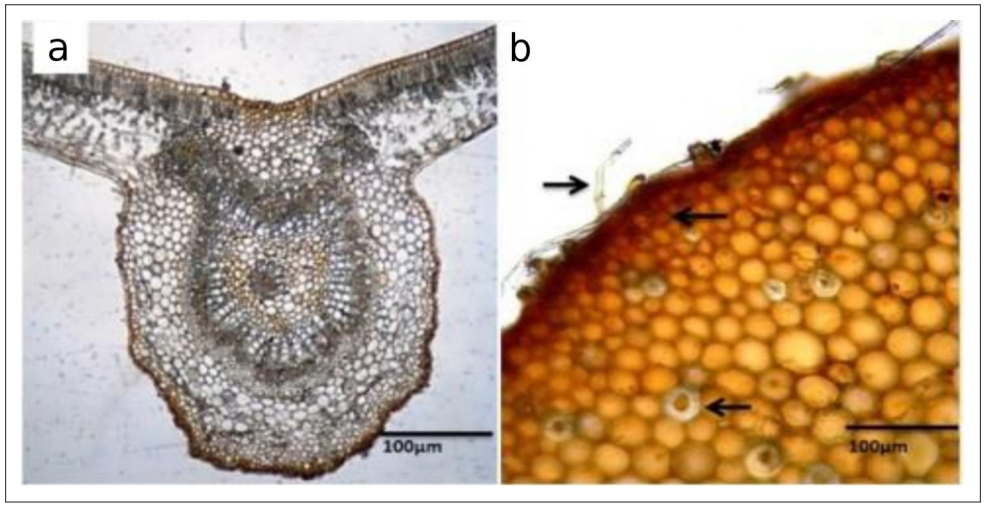

Figure 1. (a) Detail of the cross-section of the petiole of leaves, without dye. (b) Detail of the cross-section of the petiole of the leaves stained with hydrophilic vanillin, showing the presence of tannins within the tectric trichomes, and idioblasts and presence of tannins throughout the epidermal region.

association, which creates protection against herbivory.[17] While *D. macrophylla* and *D. longiflora* lack ant symbiosis, it forced them to find another way to protect themselves from herbivory and fungal pathogens.[18,19] Studies reveal alkaloid production in *D. macrophylla* leaves.[10,12,13] Our study shows *D. longiflora* presented great concentration of tannins in their leaves. We hypothesize that the production of these compounds may be related to herbivory protection, since it is known that the presence of trichomes and flavonoids production are related to insect and pest protection.[18] Also, a study reveals that production of epicatechin compounds in plants can be related to protection from fungi infections,[19] which could explain the production of this specific compound in *D. longiflora*. This work can be used as a basis for future chemotaxonomic and ecological studies.

Experimental

Plant Material

Duroia longiflora leaves were collected in three places: (1) in Reserva Florestal A. Ducke, located at Km 26 on the Manaus-Itacoatiara Road (AM-010); (2) in ZF-2 Forest Management Base, Tropical Forestry Station located at Km 50 on the BR-174, and (3) in Km 23 of ZF-2 branch, Amazonas-Brazil. A voucher specimen was deposited in the Herbarium of the Amazonas Federal Institute of Education, Science and Technology, no. 10 893.

Histochemical Study

Five healthy leaves from the third or fourth node of young shoots were collected from 3 individuals of *D. longiflora*. These were fixed in FAA70 for 48 hours and stored in 70% alcohol before being submitted to histochemical tests. Material was sectioned with a rotary table microtome blade adapted to a free hand cut microtome and placed in a watch glass where it was stained with ferric chloride[20] to phenolic substances and hydrochloric vanillin[21] to tannins, where brown and red color indicated the presence of substances. Sections were placed on a glass slide and analyzed with a microscope (Zeiss Primo Star –Microimaging 37081) at 100 × 10 magnifications. Material revealed in leaf petiolar and laminar tissues were then photographed under a microscope coupled to a digital camera (Canon PC1252).

Extracts Preparation

Leaves were dried in a forced circulation oven (30°C-40°C), grounded in a knife mill (Tecnal, model Willye TE-650). Plant material was extracted with hexane and ultrasonic bath (Unique) for 20 minutes, then filtered and extracted two more times. Then, plant material was oven-dried and extracted with methanol and finally, extracted with distilled water, by the same procedure. Hexane and methanolic extracts were concentrated in a rotary evaporator (Fisatom, model 802), while aqueous extracts was lyophilized (CHRIST, model Beta 1-8 LD plus).

Chromatographic Fractionation

Methanol extract (10 g) diluted in 1:1 methanol/H_2O and partitioned with dichloromethane (DCM), repeated three times. The hydroalcoholic solution was then extracted with ethyl acetate, also 3 times. This procedure yielded 1.25 g of DCM phase, 7.9 g of ethyl acetate phase, and 1.97 g of hydroalcoholic phase. Based on prior thin-layer chromatography (TLC) analysis, the DCM phase was selected for fractionation, because it showed to be rich in tannins (flavonoids) and showed the best

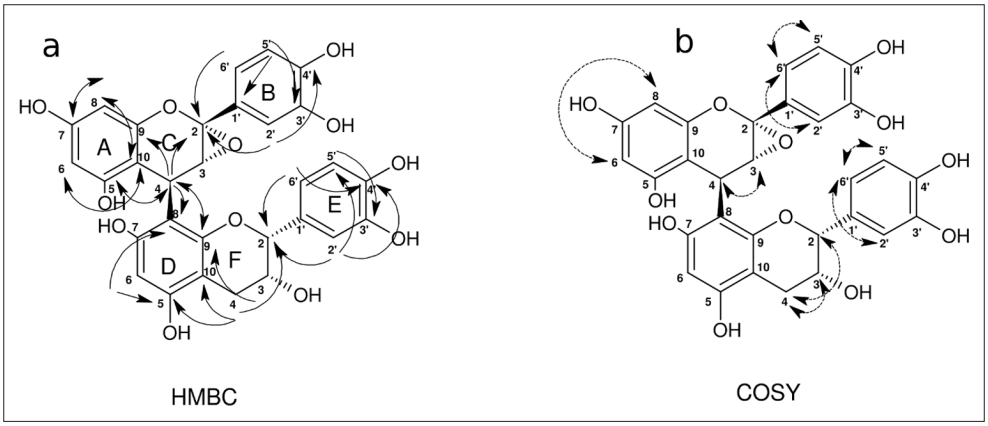

Figure 2. Main heteronuclear multiple bond correlation (a) and correlation spectroscopy (b) of 2α,3α-epoxy-5,7,3′,4′-tetrahydroxyflavan-(4β→8)-epicatechin.

separation condition in ethyl acetate/methanol 9:1 as eluent. The DCM phase of the leaf methanolic extract (1 g) was fractionated in a Sephadex LH-20 column using isocratic methanol, obtaining 64 fractions which were concentrated in a rotary evaporator, analyzed by comparative TLC and revealed with the chemical reagents (sulfuric anisaldehyde and ferric chloride) and also UV lights (254 and 365 nm). After reunion, subfraction 5 to 12 (0.2844 mg) was fractionated using silica gel (60 Mesh) as a stationary phase and increasing gradient of the solvents, in binary combinations: DCM/ethyl acetate and ethyl acetate/methanol, resulting in 33 fractions which were mixed after TLC analysis. Sub-fraction 31 to 32 (18 mg) showed absorption at 254 nm when analyzed by TLC, a purple spot when stained with ceric sulfate and a blue spot when stained with ferric chloride. These information suggested the presence of a substance with an aromatic ring together with an oxidizable site on it.

Chemical identification was performed by 300 MHz NMR analysis (Bruker Biospin AG, Forrier Model 300 UltraShield), where ^{1}H, ^{13}C, heteronuclear single quantum coherence, heteronuclear multiple bond correlation, correlation spectroscopy, and *J*-res nuclear magnetic resonance experiments were obtained. The sample was also analyzed by high resolution mass spectrometry (electrospray ionization, using micrOTOF instrument, Bruker model), generating a *quasi*-molecular ion [M + 1] at 577.1399 *m/z*, which allowed determination of the molecular formula as $C_{30}H_{24}O_{12}$.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

References

1. Barreiro EJ, Bolzani VS. Biodiversidade: fonte potencial para a descoberta de fármacos. *Química Nova*. 2009;32(3):679-688.
2. Rubiaceae in Flora do Brasil 2020 under construction. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/ FB210. Accessed March 11, 2018.
3. Martins D, Nunez CV. Secondary metabolites from Rubiaceae species. *Molecules*. 2015;20(7):13422-13495.
4. Delprete PG. Parte 1: Introdução, Gênero A-H. In: *Flora dos Estados de Goiás e Tocantins*. Goiânia: Coleção Rizzo; 2010:580.
5. Nascimento JM. Duroia L. f. (Gardenideae – Rubiaceae) na Amazônia Brasileira. In: *Dissertação de Mestrado, Universidade Federal Rural da Amazônia*. Belém, Pará; 2013:79.
6. Aquino R, Tommasi ND, Tapia M, Lauro MR, Rastrelli L. New 3-methyoxyflavones, an iridoid lactone and a flavonol from *Duroia hirsuta*. *J Nat Prod*. 1999;62(4):560-562.
7. Page JE, Madriñán S, Towers GHN. Identification of a plant growth inhibiting iridoid lactone from *Duroia hirsuta*, the allelopathic tree of the "Devil's garden". *Experientia*. 1994;50(9):840-842.

8. Carrion LL, Ramos DF, Martins D, et al. Antimycobacterial activity of Brazilian Amazon plant extracts. *Int J Phytomed*. 2013;5(4):479-485.
9. Martins D, Carrion LL, Ramos DF, et al. Triterpenes and the antimycobacterial activity of *Duroia macrophylla* Huber (Rubiaceae). *Biomed Res Int*. 2013;2013:1-7.
10. Martins D, Fachin-Espinar MT, Oliveira TA, et al. Phytochemical prospection and biological activity of *Duroia macrophylla* (Rubiaceae). *J Pharm Pharmacogn Res*. 2014;2(6):158-171.
11. Mesquita DWO, Mesquita ASS, Cursino LMC, et al. Atividades biológicas de espécies amazônicas de Rubiaceae. *Rev Bras Plantas Med*. 2015;17(4):604-613.
12. Nunez CV, Santos PA, Roumy V, et al. Raunitidine isolated from *Duroia macrophylla* (Rubiaceae). *Planta Med*. 2009;75(9):1037-1037.
13. Nunez CV, Roumy V, Mesquita DWO, et al. Indole alkaloids from *Duroia macrophylla* (Rubiaceae). *Planta Med*. 2012;78(11):1209-1209.
14. Reis AJ, Carrion LL, Rodrigues K, et al. Avaliação das atividades antifúngica, antimicobacteriana e larvicida de *Duroia macrophylla* e *D. saccifera*. *Revista Epidemiol Control Infecção*. 2016;1:108-124.
15. Wang L, Lou G, Ma Z, Liu X. Chemical constituents with antioxidant activities from Litchi (*Litchi chinensis* Sonn) seeds. *Food Chem*. 2011;126(3):1081-1087.
16. Yang C-Y, Ha W, Lin Y, Jiang K, Yang J-L, Shi Y-P. Polyphenols isolated from *Xanthoceras sorbifolia* husks and their anti-tumor and radical-scavenging activities. *Molecules*. 2016;21(12):1694-1708.
17. Frederickson ME, Gordon DM. The intertwined population biology of two Amazonian myrmecophytes and their symbiotic ants. *Ecology*. 2009;90(6):1595-1607.
18. War AR, Paulraj MG, Ahmad T, et al. Mechanisms of plant defense against insect herbivores. *Plant Signal Behav*. 2012;7(10):1306-1320.
19. Ferreira RB, Monteiro S, Freitas R, et al. Fungal pathogens: the battle for plant infection. *Crit Rev Plant Sci*. 2006;25(6):505-524.
20. Johansen DA. *Plant Microtechnique*. New York, NY: McGraw-Hill; 1940.
21. Mace ME, Howell CR. Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings. *Can J Bot*. 1974;52(11):2423-2426.