SciMedCentral
JSM Environmental Science & Ecology

Review Article

Phytochemistry of the Fruit of the Critically Endangered Tree *Pleodendron Costaricense* (Canellaceae)

Serena Bezanson[1], Sharon Curtis[1], Melissa Mata-Quiros[2], Maria Jose Mata-Quiros[3]* and Tony Durst[1]*

[1]*Department of Chemistry and Bimolecular Sciences, University of Ottawa, Canada*
[2]*Universidad International de las Americas, San Jose, Costa Rica*
[3]*OSA Conservation, Puntarenas, Costa Rica*

***Corresponding author**
Tony Durst, Department of Chemistry and Bimolecular Sciences, University of Ottawa, Canada

Submitted: 23 May, 2024
Accepted: 14 June, 2024
Published: 17 June, 2024
ISSN: 2333-7141

Keywords
- *Pleodendron Costaricense* Fruit
- Essential Oils
- Drimane Dialdehydes
- Capuchins

Abstract

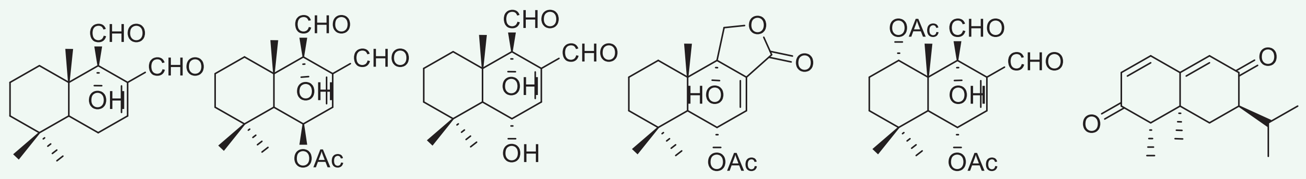
The phytochemicals found in the fruit of the extremely rare *Pleodendron costaricense*, are reported. Both the peel and the pulp contain mixtures of oxygenated essential oils and drimane dialdehydes, which are known to have a variety of biological activities. The major constituent in the essential oil is β-farnesene accounting for 75% of the essential oils in the pulp. Cinnamodial and mukaadial are the major dialdehydes found in the peel and pulp, respectively. The question of why capuchin monkeys bite into the fruit and rub the contents in the pulp on their fur but do not eat the fruit is addressed. The glycerides in the seeds consist of two unsaturated (oleic, 53.5%, and linoleic acid, 25.9%) and two saturated acids (palmitic, 14%, and stearic, 6.5%) acids. This work may help shed light on reasons why these trees are only very rarely reproducing naturally.

[#]Dedicated to Luis Poveda Alvarez, and the memory of Pablo Sanchez Vindas, both outstanding botanists. They introduced us to the two *Pleodendron costaricense* trees growing in the mountains south of Parrita, Costa Rica.

INTRODUCTION

The Canellaceae family of trees consists of six genera and sixteen species. It is found in Madagascar, tropical Eastern Africa, South and Central America, and on some of the Caribbean Islands. Canellaceae is one of the most threatened angiosperm families. One member of this family, *Pleodendron costaricense* N. Zamora Hammel & Aguilar is an extremely rare rainforest tree that has an IUCN Red List rating of Critically Endangered Species [1]. The species was discovered in 1998 during the building of an access road to a hydroelectric project in the mountains south and east of the town of Parrita on the south-west coast of Costa Rica. At that time, only two mature examples of this tree were known. The trees were described, categorized, and named by Hamel and Zamora [2,3]. These authors described it as a living fossil belonging to the earliest angiosperms originating, based on pollen records, in the Oligocene Period that lasted from about 33.9 to 23 million years ago. Another member of the family, *Pleodendron marcanthum* [4], native to Puerto Rico is al-so rare and threatened with extinction.

We reported a phytochemical investigation of the leaves and bark of the newly dis-covered and extremely rare *Pleodendron costaricense* (*Canellaceae*) tree in 2002 [5]. We found that the fragrant leaves produced small amounts of essential oils reminiscent of pepper and cinnamon consisting mainly of α- and β-pinene, limonene, and caryophyllene. The other major constituents in the leaves and the bark belonged to the drimane family. These included polygodial, 1, cinnamodial, 2, also known as ugandesidial [6], mukaadial, 3, cin-namosmolide, 4, compounds typically found in plants of the Canellaceae family (Figure 1). Two new compounds parritadial 5, and pleodendione, 6, were also described. We reported that 1 had potent activity against human fungal pathogens *Canadida albicans* (wild type and azole-resistant forms) *Candida shehatae*, *Cryptococcus neoformis*, *Wangiella dermatitidis*, and the phytopathogen *Alternaria alternata* [5]. Compounds 2, 5 and 6 had weaker antifungal activity. Antifungal activity has been reported by other authors [7,8]. These dialdehydes show a variety of other useful biological activities. Strong antifeedant activity against African armyworms has been reported for cinnamodial 9 and warbuganal [8,10]. Cinnamo-dial is also a mosquito (*Aedes aegypti*) repellant [10]. Polygodial displays a diverse range of biological activity including antifouling, insect antifeedant or deterrent, antibacterial, antiphrastic, anti-fungal, anti-allergic, and anti-inflammatory activity [11]. They are hot tasting to humans. *Warburgia* tree material has been used as a food spice [12].



1, polygodial **2**, cinnamodial **3**, mukaadial **4, cinnamosmolide** **5**, parritadial **6**, pleodendione

Figure 1 Drimane sesquiterpenes from *Pleodendron costaricense.*

Scientists at the Costarican NGO Osa Conservation have recently confirmed the existence of an additional seven mature trees of this species in two separate forest patches in the Osa Peninsula. They have not been able to discover any naturally growing seedlings but have succeeded in producing close to one hundred and fifty seedlings in their nursery. The germination rate was accelerated by exposing some of the many small shiny black seeds of the ripe fruit to gibberellic acid. Some of the nursery-produced seedlings have been transplanted into the nearby forest; they have survived for over two years and are growing well. The natural slow germination coupled with high levels of predation by ants and cockroaches seems most likely to contribute to the absence of natural regeneration of the species.

Cameras were installed both in these trees and on the nearby ground to monitor potential seed distributors. During this study, it was noticed that white-faced capuchin monkeys (*Cebus imitator*) frequented these trees and collected some fruit. They would bite into the fruit and then rub themselves and each other with the essential oils and other phytochemicals that were in the peel and the pulp of the fruit. They did not consume the fruit [13]. The question is: Why do they do this? One obvious possibility is that the fruit peel and pulp constituents have insect-repellant properties. Another possibility is that these monkeys find the fragrance attractive. Finally, do they have fungal infections and find that this activity reduces the unpleasantness associated with such infections?

METHODS

Fruit, approximately 625 g, was harvested during July and August 2022 (Voucher number LAA303, National Herbarium of Costa Rica CR, collected under permit from SINAC No SINAC-ACOSA-D-PI-R-046-2022) and kept frozen for five months. It was thawed and separated into peels, watery pulp, and seeds. The seeds were allowed to dry. The peel and pulp were sterilized with 95% ethanol and then water was added to produce a 1:2 mixture suitable for safe transport. The latter materials were stored in a -10C freezer until they were further processed.

Extraction of the Peels and Pulp of P. Costaricense

The mixture containing the peels was diluted with two volumes of water and then filtered to remove the insoluble peels. The filtrate, 300 mL was extracted first with 100 mL of hexane and then with 100 mL of dichloromethane. The organic phase was dried with an-hydrous magnesium sulfate and the solvents were evaporated under reduced pressure. The extracts, approximately 100 mg with each solvent, were examined via GC/MS.

The peels were dried at ambient temperature and then finely ground with a small coffee grinder to give 65 g of product. Portions of the ground material were extracted by stirring with dichloromethane at room temperature for 2 h. This afforded a brownish oil with about 6-7% yield by weight.

Extraction of the pulp mixture, (total volume 1 L after dilution after dilution with an additional 500 mL of water), with ethyl acetate was greatly complicated by the formation of a persistent emulsion, and only small amounts of extract were obtained even when extracting with several hundred mL of solvent since only a small volume of the organic sol-vent separated from the emulsion. This material was not further investigated. The aqueous phase was filtered by gravity and the remaining solids were allowed to dry in an open vessel. The yield of dry solids was 122 g. This material, which had a pleasant peppery odor, was then ground into a powder. It was extracted by stirring at room temperature for two hours with dichloromethane.

Extraction of the P. Costaricense Seeds

The dry seeds were ground in a mortar with a pestle and then extracted by stirring with a five-fold amount of either 95% ethanol or dichloromethane at room temperature for 2 h.

RESULTS

Chemical Constituents in the Peel

Extraction of the aqueous fraction in which the peels had been stored with hexane gave a small amount of an oil that contained mainly the dialdehydes cinnamodial, 2 (64.9%), polygodial, 1 (7.8%) and parritadial, 5, (4.5%). The remainder consisted mainly of monoterpenes including thujene, pinenes, β-myrcene, linalool, and terpineol. Re-extraction of the aqueous phase with dichloromethane gave approximately 100 mg of a mixture containing again mostly cinnamodial (63%), parritadial (15%) polygodial (8%); this fraction also contained small amounts of hydrocarbon monoterpenes. Extraction of the dried peel with dichloromethane at room temperature for 2 h afforded a brownish oil with a 6% yield. GC/MS analysis gave results similar to those described above.

Chemical Constituents in the Dried Pulp

The aqueous portion of the pulp as described above yielded only about 0.5 g of organic soluble material whose major components, based on gc/ms were the aldehydes polygodial (10.5%) warburganal (8.9%), parritadial (8.3%), and cinnamodial (6.9%). This mixture was not further investigated. In contrast, extraction of 30 g of dried, finely ground pulp yielded 7.5 g of light brownish oil after stirring for 90 min at room temperature with a five-volume-to-weight ratio of dichloromethane. Chromatography of 5.0 g of this material on 100 g of silica gel gave on elution with 95:5 hexane-ethyl acetate 250 mg of nonpolar mono- and mainly non-oxygenated sesquiterpenes. The ten components representing 98% of this fraction were identified and quantified by GC/MS (Table 1). E-β-Farnese represented more than 75% of this fraction.

Continued chromatography with 200 mL of 9:1 hexane ethyl acetate gave fractions that contained mixtures of oxygenated with the major identified oxygenated terpenes being nerolidol, drimenol, and α-cadinol. Cinnamosmolide, 4, was also present in these fractions. Elution with 200 mL of 2:1 hexane-ethyl acetate gave fractions whose major components were identified by a combination GC/MS and ^{1}H NMR as the antifungal drimane aldehydes, cinnamodial, mukaadial, and parritadial all previously isolated from the leaves and bark [3] Mukaadial, 0.65 g, was most abundant. It was obtained in pure form as a white solid. The combined weight of the fractions eluted with this mixture was 2.2 g. A 400 MHZ ^{1}H NMR of the total crude extract indicated, based on peaks in the 9.4-9.9 ppm region of the spectrum, the presence in small amounts of at least four additional unidentified dialdehydes.

Chemical Constituents in the Seeds

Extraction of the seeds with either dichloromethane or 95% ethanol afforded a mixture of triglycerides in close to 20% yield. The NMR spectra of these extracts were identical and indicated the presence of considerable amounts of unsaturation in the fatty acid components. The mixture was hydrolyzed by heating overnight in methanol containing an equal amount of 10% sodium hydroxide. The reaction mixture was then cooled, acidified with 10% HCl solution, and extracted with ethyl acetate. This afforded a mixture of fatty acids in excellent yield as shown by ^{1}H NMR. This mixture was esterified by refluxing in a 10:1 mixture of methanol-conc. sulfuric acid solution for 3 h. The resultant mixture of methyl esters obtained in more than 90% yield was analyzed by GC/MS. This analysis showed that the P. costaricense glycerides consisted of two saturated fatty acids: stearic (6.5%) and palmitic acid (14%), the monounsaturated oleic acid (25.9%) and doubly un-saturated linoleic acid (53.6%). The fatty acid composition in the glycerides found in the seeds of P. costaricense is roughly comparable to that of corn or cottonseed oil which are reported to consist of about 58% linoleic and 20% or less of saturated acids [10].

Table 1: Non-polar components obtained from the dried *P. costaricense* pulp.

Compound	(%)	Compound	(%)
E-β-farnesene	75.2	β-myrcene	8.8
β-pinene	4.2	Z,E-α-farnesene	2.8
b-caryophyllene	2.5	β-phellandrene	2.2
α-ocrimene	1.5	E,E-α-farnesene	0.98
α-cubebene	0.94	α-copaene	0.86

DISCUSSION

Both the peel and the pulp of the *P. costaricense* fruit contain a mixture of unoxygenated mono-and sesquiterpenes together with smaller amounts of oxygenated sesquiterpenes. The major components in both parts of the fruit are the mixtures of the highly bioactive drimane dialdehydes: cinnamodial, mukaadial, warburganal, and parritadial, with cinnamodial and mukaadial being the major aldehyde in the peel and pulp, respectively. These compounds are also present in the leaves and bark of the tree [5]. The secondary metabolites account for close to 25% of the total weight of the dried pulp. Surprisingly, the pulp does not contain significant amounts of flavonoids or other soluble secondary metabolites. It contains only minor amounts of triglycerides; we identified the presence of no more than 10 mg of ethyl palmitate and palmitic acid in one of the intermediate polarity fractions. The insoluble material is presumably a mixture of polysaccharides, starch, and cellulose. Except for the potential of the insoluble portion of the pulp, there appears to be surprisingly little nutritional value in the pulp.

The drimane dialdehydes have been reported to be hot to the taste [12]. Is this why the capuchin monkeys do not consume, but only bite into the fruit to release the combination of the essential oils and the dialdehydes in the pulp, and rub this material on their fur and the fur of other individuals? This is known as social anointing and has been described several times in *Cebus* and *Sapajus* monkeys [14]. Such behavior is hypothesized to provide chemical defense against ectoparasites, to provide olfactory communication cues, and to be a social bonding activity. The three functions are not mutually exclusive.

Cinnamodial and polygodial have been shown to act as antifeedants and to repel mosquitoes [10,11]. Most likely the monkeys rub themselves with the aromatic broken fruit to release insect repellents and insect antifeedants. Alone, or in combination, including with the other dialdehydes, these compounds have significant anti-fungal activity against human and primate skin pathogens causing thrush (*Candida spp.*) and more serious systemic infections by *Cryptococcus neoformans* and *Wangiella dermatitidis*. They could therefore provide treatment for or protection against fungal infection [5,7-9]. Their minty and slightly peppery odor might also be appealing to the capuchins.

The natural slow germination, coupled with high levels of predation by ants and cockroaches, are suggested to be major reasons for the absence or very low level of natural regeneration of the species. It seems that an increase in seed predators is a more likely cause than the germination rate which is not expected to have changed significantly over long periods. Perhaps, there is also a decrease in seed distributors, especially those whose digestion might reduce germination time. The seedlings produced by the Osa Conservation Authority seem to survive and thrive in the natural setting indicating that the absence or very low rate of natural germination, and not their destruction is the major cause of the scarcity of this species.

ACKNOWLEDGMENTS

The authors would like to thank Prof. Ana Frances Carballo, UNA, Heredia, Costa Rica for storing the fruit and providing the solvents required for their safe transportation and Prof. J. T Arnason for useful comments and editing of the manuscript.

AUTHOR CONTRIBUTIONS

The project was conceived, and the manuscript was written with contributions from all authors. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Rivers MC. Pleodendron costaricense. The IUCN Red List of Threatened Species 2019.

1. Salazar J, Nixon K. New discoveries in the Canellaceae in the Antilles: How Phylogeny can support Taxonomy. Botanical Review. 2008; 74: 103-111.

2. Hammel BE, Zamora NA. Pleodendron costaricense (Canellaceae), a new species for Costa Rica. Lankesteriana. 2005; 5: 211-218.

3. US Fish, Wildlife Service. Recovery plan for Pleodendron macranthum and Eugenia haematocarpa. Atlanta. 1998.

4. Treyvaud Amiguet V, Petit P, Ta CA, Nuñez R, Sánchez-Vindas P, Alvarez LP, et al. Phytochemistry and antifungal properties of the newly discovered tree Pleodendron costaricense. J Nat Prod. 2006; 69: 1005-1009. doi: 10.1021/np0504863. PMID: 16872133.

5. Brooks CW, Draffan GH. Tetrahedron. 1969; 25: 2887-2898.

6. Taniguchi M, Adachi T, Haraguchi H, Oi S, Kubo I. Physiological activity of warburganal and its reactivity with sulfhydryl groups. J Biochem. 1983; 94: 149-154. doi: 10.1093/oxfordjournals.jbchem.a134323. PMID: 6352692.

7. Kubo I, Himejima M. Potentiation of antifungal activity of sesquiterpene dialdehydes against Candida albicans and two other fungi. Experientia. 1992; 48: 1162-1164. doi: 10.1007/BF01948015. PMID: 1473583.

8. Kubo I, Miura I, Pettei MJ, Lee YW, Pilkiewicz F, Nakanishi K. Muzigadial and warbuganal, potent antifungal, anti-yeast, and African Armyworm antifeedent agents. Tetrahedron Letters. 1977; 18: 4553-4556.

9. Kalsi M, Walter A, Lee B, DeLaat A, Trigueros RR, Happel K, et al. Stop the crop: Insights into the insecticidal mode of action of cinnamodial against mosquitoes. Pestic Biochem Physiol. 2021; 171: 104743. doi: 10.1016/j.pestbp.2020.104743. Epub 2020 Nov 8. PMID: 33357565; PMCID: PMC7770332.

10. Kubo I, Lee YW, Pettei M, Pilkiewicz F, Nakanishi K. Potent armyworm antifeedants from the East African warburgia plants. Journal of the Chemical Society Chem. Comm.1976; 1013-1014.

11. Kubo I, Ganjian I. Insect antifeedant terpenes, hot tasting to humans. Experientia. 1981; 37: 1063-1064. doi: 10.1007/BF02085009. PMID: 7308389.

12. Messer E J, Bowler MT, Claidière N, Whiten A. The role of anointing in robust capuchin monkey, Sapajus apel-la, social dynamics. Animal Behaviour. 2022; 190: 103-114.

13. Alfaro JW, Matthews L, Boyette AH, Macfarlan SJ, Phillips KA, Falótico T, et al. Anointing variation across wild capuchin populations: a review of material preferences, bout frequency and anointing sociality in Cebus and Sapajus. Am J Primatol. 2012; 74: 299-314. doi: 10.1002/ajp.20971. Epub 2011 Jul 18. PMID: 21769906.