Project Rapid Field Identification of *Dalbergia* Woods and Rosewood Oil by NIRS Technology – NIRS ID.

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HISTORICAL AND GENERAL OVERVIEW OF TECHNICAL-SCIENTIFIC PRODUCTION ON ROSEWOOD OIL (Aniba rosiodora Ducke)

Consulting objectives: OBTAIN A HISTORICAL AND GENERAL OVERVIEW OF TECHNICAL-SCIENTIFIC PRODUCTION ON ROSEWOOD OIL (*Aniba rosiodora* Ducke) FROM THE AMAZON FOREST, SPANNING TOPICS SUCH AS ECOLOGY, ECONOMY, TECHNOLOGY, GEOGRAPHY, METHODS OF OBTAINING OIL AND CONVENTIONAL CHEMICAL ANALYSIS TO QUALIFY THE PRODUCT.

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1 INTRODUCTION

The project "Project Rapid Field Identification of *Dalbergia* Woods and Rosewood Oil by NIRS Technology" aims to support the conservation of Amazonian biodiversity, especially, of species listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The project features the NIRS Technology (association of near-infrared spectroscopy and multivaried analysis) to wood identification as a great potential tool, which has already been proven in several publications (Silva et al. 2018, Snel et al. 2018, Soares et al. 2017, Bergo et al. 2016, Braga et al. 2011, Pastore et al. 2011), to manage, monitor and control wildlife threatened species by trade.

The project aims two fundamental goals: 1) develop models to identify wood from 20 species of genus *Dalbergia* and 2) develop an exploratory model to identify the oil extracted from forest species *Aniba rosiodora*. This report concerns to contextualize the second goal, forming a historical and general overview of technical scientific production on rosewood oil from the Amazon Forest.

The greatest interest regarding to the species *Aniba rosiodora* is the extraction of its essential oil which is highly valued. In 2019, one liter of rosewood oil was estimated at US$ 350 in the international trade, according to the Amazonas State Industry Center (CIEAM). Nowadays Brazil is the only exporting country of this product in the world that follows all CITES regulations, despite the species *Aniba rosiodora* occurs in other countries of Amazon Forest region, such as: French Guiana, Suriname, Guyana, Venezuela, Peru, Colombia and Ecuador.

The rosewood predatory exploitation over the years resulted in a threatened species, especially because of the conventional exploitation practices, which imply the destruction of the entire tree. It is estimated that half a million trees have been cut down since the beginning of the exploitation in the 1930s (UNICAMP Journal 2002).

Currently, the species *Aniba rosiodora* has been listed in Appendix II of CITES since 2011. International trade in specimens of Appendix-II species may be authorized by the granting of an export permit or re-export certificate. Moreover, *Aniba rosiodora* Ducke is also listed in The Red List of Brazilian Flora (CNCFlora 2012), the Official List of Endangered Species of the Brazilian Flora elaborated by Ministry of Environment of Brazil (Regulation nº 443 of 12/17/2014) and the red-list created by International Union for Conservation of Nature (IUCN) where *Aniba rosiodora* was included in endangered category (IUCN 2014).

Therefore, to preserve and maintain the species, it's essential to develop methodologies and tools that control the exploitation. One of these methodologies is the

NIRS technology that is used to identify wood and also, some other alternatives methods are used to the extraction of *Aniba rosiodora*'s product.

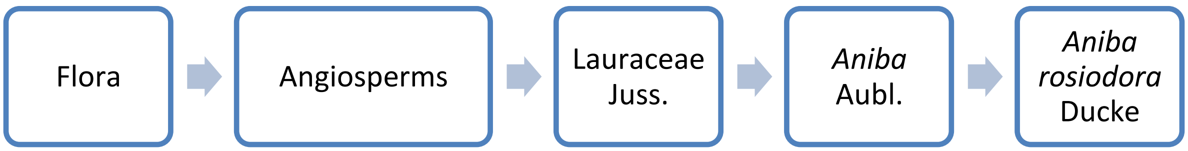
2 APPLIED METHODS

The consultancy was based on bibliographic searches that were made by online consultations of documents that aim to attention the rosewood oil. The research was performed by fast and advanced searches in the main scientific and non-scientific database (Science Direct, World Wide Science, Web of Science, Google Scholar, National Institute for Amazonian Research, and others), with the access and classification of the available files dealing directly on the species *Aniba rosiodora*.

3 RESULTS

3.1 BOTANICAL DESCRIPTION AND PHYTOGEOGRAPHY OF THE SPECIES *Aniba rosiodora*

The taxonomic hierarchy of the species *Aniba rosiodora* is described in REFLORA Programme according to the following flowchart:



Flowchart 1: Taxonomic hierarchy of the species *Aniba rosiodora* Ducke, Brazilian Flora 2020, accessed Januray 22th, 2021, <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB78444>.

Lauraceae, or the laurel family, is a family of great representativeness in Amazonia. Besides the ecological importance, the laurel family is very commercially exploited for producing essential oils, having a good quality wood and being used in gastronomy, such as bay leaves and avocado (Nogueira 2015). Kubitzki and Renner (1982) review about genus *Aniba* recognized 41 species divided into two groups according to the structure of stamen: *affinis* and *guianensis*.

Whitin the group *guianensis*, there is a subgroup called *panurensis*, which rosewood *Aniba rosiodora* Ducke is part of along with other species: (*Aniba panurensis* (Meisn.) Mez, *Aniba firmula* (Nees & Mart.) Mez, *Aniba parviflora* (Meisn.) Mez, *Aniba rosiodora* Ducke, *Aniba cylindriflora* Kosterm., *Aniba heringeri* Vattimo-Gil, *Aniba coto* (Rusby) Kosterm. and *Aniba muca* (Ruiz & Pav.) Mez.). These species are indistinguishable based on vegetative character and they form a subgroup named **panurensis complex** by Barbosa (2015).

Moreover, the popular rosewood comprised two species, both belonging to the laurel family: *Aniba rosiodora* Ducke and *A. duckei* Kostermans (synonym for *Aniba rosiodora* var. *amazonica*). Based on biochemical evidences and morphology similarities, Kubitzi and Renner incorporated *Aniba duckei* in *Aniba rosiodora*.

The species was described originally by Ducke (1930) as *Aniba rosaeodora,* however, the spelling alteration of specific epithet *rosaeodora* to *rosiodora* was made by Brazilian Flora (Brazilian Flora 2020, <www.reflora.jgrj.gov.b>r) and Missouri Botanical Garde

(<https://www.tropicos.org/name/17802277>). The alteration was based on Melbourne Code Art. 60.10, recommendation 60 G.1, where they recommend that a name or epithet that combines elements derived from two or more Greek or Latin words should be formed, as far as practicable, in accordance with classical usage. The cited article can be found in the website of International Association for Plant Taxonomy (IAPT) <https://www.iapt-taxon.org/melbourne/main.php?page=art60>.

Whereas commercialized nationally and internationally rosewood oil is the object of this work, it shall be followed the Brazilian Flora nomenclature as *Aniba rosiodora* found in Brazilian Flora 2020 <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB78444>. In this study, the similar forest species and oil producers will be mentioned generally as *Aniba rosiodora*.

According to CITES Wood ID, *Aniba rosiodora* wood is distributed geographically in South America in the countries: Brazil (Amapá, Amazonas and Pará), Colombia, Ecuador, Guianas, Peru and Suriname (Richter et al. 2019).

The species *Aniba rosiodora* may have more than one regularly used name depending on the region where it is found: “pau-rosa”, “pau-rosa itaúba”, “pau-rosa mulatinho”, “pau-rosa imabaúba” (Brazil), “cara-cara”, rosewood (Guyana), “bois-de-rose”, “bois-de-rosa-femelle” (French Guiana), “enclit-rosenhout” (Suriname), “palorosa”, “palo-de-rosa” (Colombia), “palo-de-rose” (Peru) (Reis et al. 2014, López et al. 2015). In other countries outside of South America, it may be called as “rosenholzbaum” (Germany), brazilian rosewood, rosewood tree and “legno di rose” (Italy) (Ministerio del Ambiente Perú 2015).

The species originates in the Amazon Forest and occurs in French Guiana, distributed along the Guianas, Suriname and Amazon region of Venezuela, Colombia and Peru. In Brazil, the species is found in the northeastern Amazon rainforest, on the banks of Amazonas river and in northeastern Peru (Sudam 1972), but also in central south region of Pará and in Purus river basin located in southern Amazonas state (Mitja and Lescure 1996). Figure 1 below presents in red the species *Aniba rosiodora* geographical ranges in states of Brazil in 2012 when it was drawn up by CNCFlora. This map provides a spatial representation of the distribution of the species, based on records of botanical samples available:

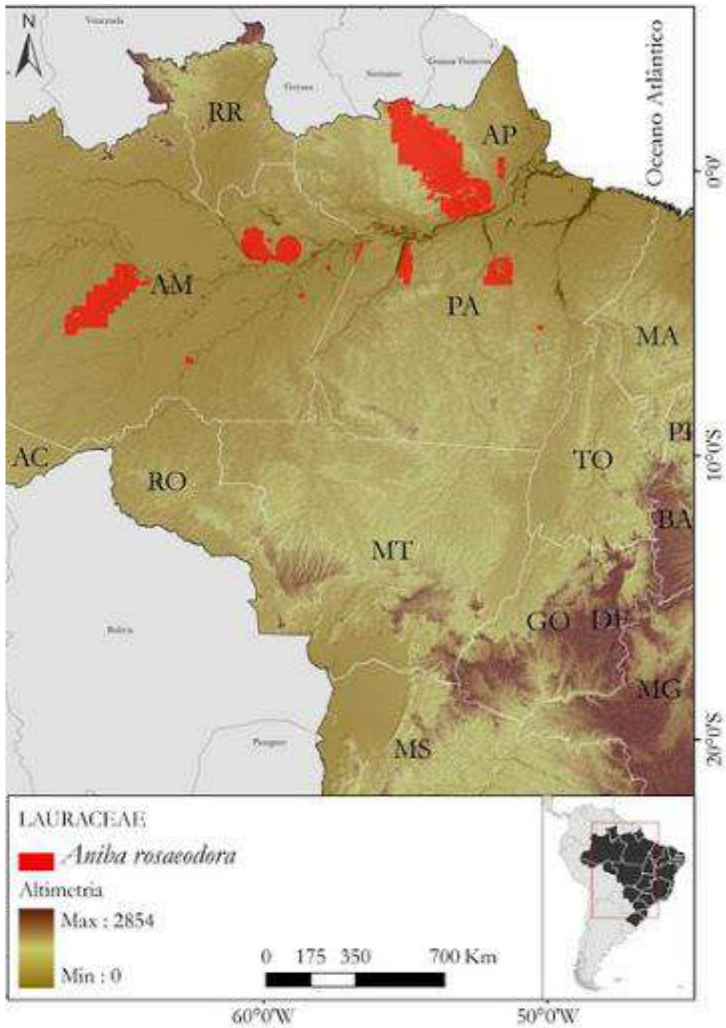


Figure 1: Geographical ranges of the species *Aniba rosiodora* in states of Brazil (CNCFlora 2012).

This species can be found in rainforest but also in "Campinarana". The name "Campinarana" refers to a vegetation type of the Amazon region, typical in humid climate, on sandy, predominantly hydromorphic soils, also called white sand vegetation (Anderson 1981). "Campinarana" is characterized by a particular landscape that stands out from the surrounding rainforest. These vegetation formations are strongly influenced by hydrological seasonal cycles and by changes in groundwater levels (Mendonça et al. 2017, Kubitzki and Renner 1982, SNIF 2020).

The botanical description and phytogeography of the species *Aniba rosiodora* are based on the works of Ducke (1938), Kubitzi and Renner (1982) and Sampaio et al. (2003).

Aniba rosiodora is a large tree that sometimes reaches a height of 20 meters and a diameter of 2m. It has a straight and cylindrical trunk and a yellowish-brown bark or reddish bark that comes off easily in large pieces. Figure 2 below presents young trees of rosewood:

Figure 2: Young trees of rosewood. (Brazilian Rosewood – Essential Oils | Guide of Brazil, accessed August 6th, 2021, < https://www.oleosessenciais.org/oleo-essencial-de-pau-rosa-brazilian-rosewood/>).

Their leaves have a great variation in size, usually about 14 cm long and 5 cm wide. Leaves abovate-elliptic or lanceolate. The upper surface is glabrous, which means without hairs of any kind, coriaceous and dark green. The under surface is slightly pubescent, which means with a hairy surface, and pale yellow. Secondary veins diverge from primary vein at an angle of 45º to 60º. The petioles are thick and glabrous, measuring around 0.9 to 1.7 cm long. The leaves are distributed alternately along the lesser branches or concentrated at the tips. Figure 3 below presents the rosewood leaves.

Figure 3: Photo of the leaves of the species *Aniba rosiodora* Ducke (Maia and Mourão 2016).

The Field Museum's online Botanical Collections Database <https://collections-botany.fieldmuseum.org/> contains specimens and historical records, including a botanical collection with photos of tree leaves from different countries in South America. Figure 4 below presents leaves of rosewood tree from Suriname, Guyana, Venezuela, Peru and Brazil:

Figure 4: *Aniba rosiodora* Ducke's leaves from the following countries: (1) Suriname, (2) Guyana, (3) Venezuela, (4) Peru and (5) Brazil (Catalogue | Botanical Collections., accessed November 8th, 2020, <https://collections-botany.fieldmuseum.org/list?genus=Aniba&species=rosaeodora>.

The flower hermaphrodite is small (around 1.5 mm long) and brownish-tomentose. The perianth has 6 erect cepals all the same size or outer ones smaller. Generally, it has 9 stamens with filaments as long as those of the anthers or shorter. Generally, anthers have upward-turning valves to release the pollen. Pistil minutely tomentose. Ovary ellipsoid or ovoid, glabrous or pilose, included in the floral tube and pedicels inconspicuous and filmanets short.

The fruit is berry-like with a cup-shaped receptacle. The receptacle is chronic, thick, with outer surface rough and greenish-brown and inner surface glaborous and brown. The berry is obovoid to ovoid in shape, green when immature and turning dark violet when mature, with only one seed. The fruits are very appreciated by birds that act as predators and, potentially, as seeds dispersers. Ripening of the fruit can be visualized by dark violet in color, when they are ready to harvest by providing higher percentage and germination rate of the seeds.

The seed of *Aniba rosiodora* is ovoid, tegument thin, smooth and opaque; light brown with longitudinal dark brown grooves. Tegument brittle when dry. The seed has two cotyledons, large, convex, hard, smooth and cream-coloured. Rudimentary embryo is erect, central, near-base with 3mm long and cream-coloured. The seeds' collection under the trees, after the natural fall of the fruit, is the most usual practice. However, irregular flowering and fruiting and also fruit predation are factors limiting seed production. Figures 5 and 6 below present the different stages of rosewood's fruit and seeds:

Figure 5: Fruits and seeds of *Aniba rosiodora* Ducke, in sequence: immature fruit, ripe fruit, cut fruit showing yellow pulp and seed with tegument, seed without tegument showing two cotyledons and front view after removing one of the cotyledons showing the basal position of embryo axis (Sampaio et al. 2003).

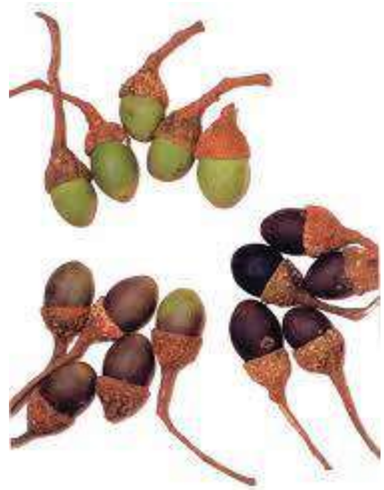


Figure 6: Rosewood's fruits in different stages of fruit development showing different color (Sampaio et al. 2003).

Aniba rosiodora stands out in the production of its essential oil that has a pleasant aroma, rich in linalool and widely used in perfume industry by retaining the fragrance. The oil is obtained from the distillation of any part of the plant, but the wood is the main source. It is worth mentioning that there are differences in yields, physic-chemical properties and oil fragrance because of the part of the plant where the oil was extracted (Zellner et al. 2006).

CITES Wood ID provides more information about *Aniba rosiodora*'s wood:

1) Growth boundaries indistinct or absent;
2) Heartwood basically brown, yellow, green without streaks;
3) Sapwood distinct from heartwood colour;
4) Odour distinct (strongly aromatic due to linalool);
5) Wood of medium weight (0.54 – 0.58 g/cm³).

Figure 7 shows some pictures of *Aniba rosiodora*'s wood in transverse section (1) and wood suface (2), both in 10x:

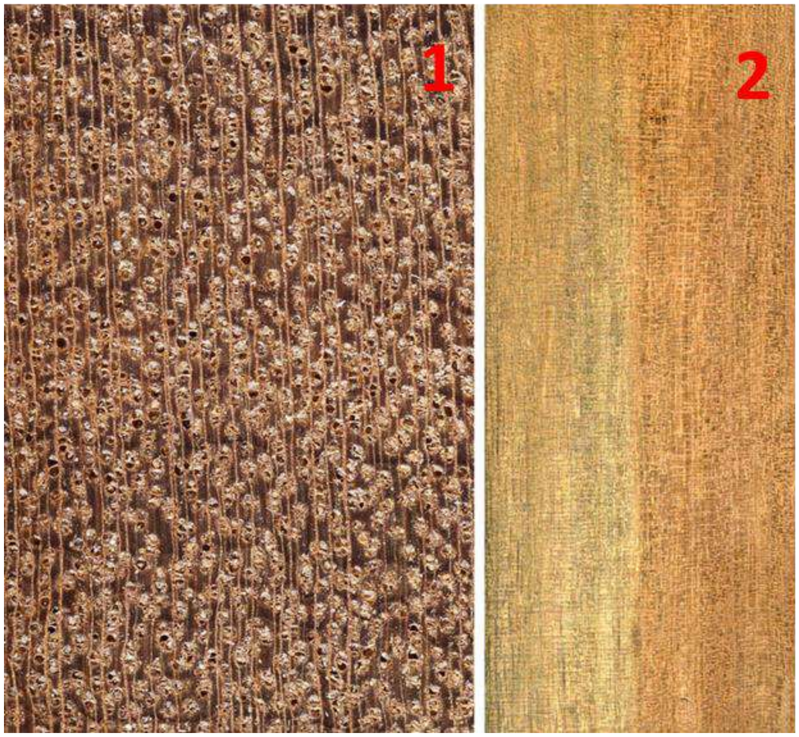


Figure 7: *Aniba rosiodora*'s wood in transverse section (1) and wood surface (2), both in 10x (Richter et al. 2019, accessed November 8th, 2020. < https://www.delta-intkey.com/citeswood/images/par.jpg>).

Figure 8 below refers to the wood of *Aniba rosiodora* and the photos were taken in the Forest Products Laboratory (LPF) in the Brazilian Forest Service (SFB):

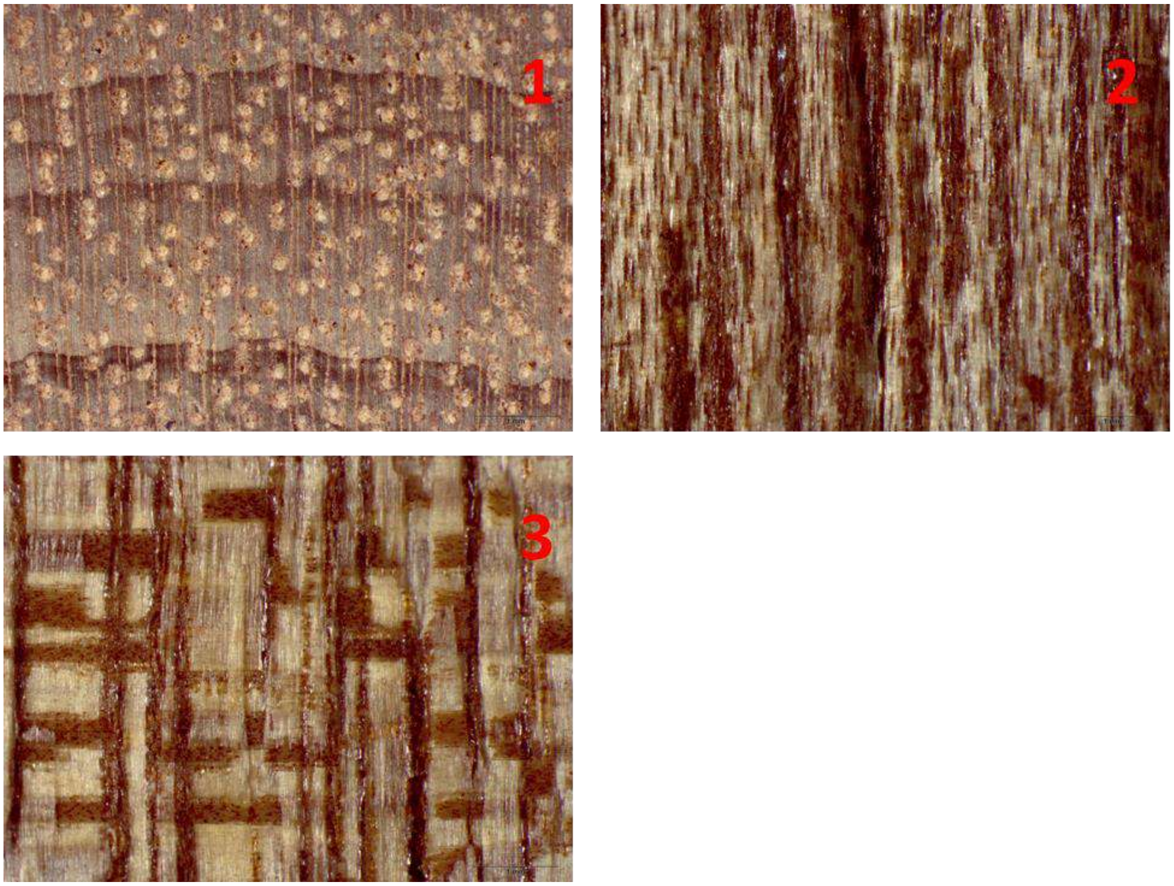


Figure 8: Wood of *Aniba rosiodora* in transverse section (1), tangential section (2) and radial section (3) (Forest Products Laboratory 2021).

3.2 EXTRACTION AND FORESTRY OF THE SPECIES *Aniba rosiodora*

The production of rosewood essential oil, despite being based on the extraction of a renewable natural resource that is wood, has become an unsustainable activity over the years because of predatory cutting of trees of the species *Aniba rosiodora* and as consequence, set the species in danger of extinction.

1) Rosewood extraction in Brazil

A brief background on rosewood extraction will be present below. The history of the extraction of rosewood oil in Brazil begins in 1920, right after the depletion of rosewood reserves in French Guiana. The processing of rosewood oil was the first agroindustry flora-chemical in Amazon region and, it exported a total of 16 tons of oil in 1926 (Homma 2003).

During the first decade of export (1920), there weren't any efforts to implement sustainable plantations of the species *Aniba rosiodora* and the harvest was arbitrary. Only in 1930 the state government of Amazonas established an amount of oil to be produced annually and the compulsory requirement to replanting for distilleries. However, the control of the extraction wasn't effective and it was unrestricted (Homma 2003).

In 1940, the trade got through a chaotic and troublesome time. The government established a set of policies to restrict the extraction and set the amount of exportations. Moreover, the Word War II affected the global trade of rosewood oil's valuation. Next decade, the amount of oil that was exploited and shipped reached the maximum of 599 tons of exploited oil and 444 tons of shipped oil (Homma 2003).

Due to intense extraction, in the decade of 1960, commercial sector started showing depletion of accessible feedstock (Homma 2003). It is worth highlighting that researches carried out by National Institute for Amazonian Research (INPA) were developed in order to study about propagation methods of the species (by seeds and stakes) and to study about techniques for full exploitation of other parts of the tree, beside the trunk, to oil extraction (Neto 1972, Araújo 1967, Araújo 1971).

In the following years, other researches were concentrated to find a natural substitute to rosewood and it was found dozen of plants that produce linalool, such as: bergamot, lemon, jasmine, basil and others (Guenther 1967). However, the use of these plants by perfume industry wasn't practicable because of its undesirable fruit fragrance and quite often these plants produced oils that could irritate airways and skin. In that way, rosewood essential oil is unique (Homma 2003).

Thinking about synthetic substitutes, American laboratories synthesized the primary components found in the oil: linalool and linalyl acetate (Gottlieb 1957). The success of the chemical synthesis could offer to global market an oil substitute at reduced prices and it has brought changes to natural product extraction in the 1970s. However, even if there was available essential oil made from the synthetic substitutes, the natural product remained highly esteemed value because of its fragrance and pleasant smell (Homma 2003).

In the late 1980s, the depletion of accessible feedstock of rosewood encouraged that Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) to edit the Ordinance 01/98 in August of 1998 that regulated the exports, industrialization and marketing of rosewood. From there on, logging with Diameter at Breast Height (DBH) less than 20 cm was prohibited (IBAMA 1998).

It's estimated that about a half million of trees of *Aniba rosiodora* were harvested since the beginning of the exploitation and IBAMA recognized *Aniba rosiodora* in the list of endangered species in 1992 (Ordinance IBAMA nº 37 of April 3rd in 1992). Moreover, the harvesting areas of the species and distillation were restricted and in 2004, those areas were limited to the cities of Parintins, Rio Madeira, Presidente Figueiredo, Manicoré and Maués, despite the existence of the species' trees in other Amazon regions (May and Barata 2004).

Nowadays, the oil extractors of rosewood are required by IBAMA to restore 80 seedlings for each 180kg-barrel exported (Ordinance nº25 of October 1st in 2010 and Normative Instruction nº9 of August 25th in 2011). This situation and inclusion of the species *Aniba rosiodora* in CITES Appendix II have increased the demand for seedlings and seeds of *Aniba rosiodora* in Central Amazon. The following figure 9 presents a seedling of *Aniba rosiodora* with 6 months:

Figure 9: Seedlings of *Aniba rosiodora* with 6 months with simple alternated-spiral leaves (Sampaio et al. 2013).

2) Forestry of rosewood

The demand for seedlings and seeds boosted the studies about natural and artificial methods for regeneration of the species *Aniba rosiodora*. The development of techniques of propagation (tissue culture, cutting and natural regeneration) became essential for commercial plantations.

Traditionally, the species can be propagated by their seeds; however, those seeds are eaten mainly by birds, rodent and bugs. This makes it difficult to get a large number of viable seeds to produce seedlings (Sampaio et al. 2003). The seeds are not always available because when the fruits ripe, the seeds escape from the treetop and fall on the ground, and thus seeds can be eaten by predators on the treetop and after dispersion. This is one of the most difficult points for the species' propagation.

It is worth mentioning that rosewood should be stored with water content above 40% because they're recalcitrant which means that cannot survive desiccation, so germinates instantly. Rosewood seeds are also intolerant to freezing; that's why storage of the seeds should be done taking into consideration these specifications (Sampaio et al. 2003). Therefore, there's a need for developing techniques and improvements to obtain rosewood seedlings.

The seedling can be produced by direct sowing in individual plastic bags or in a sowed area to subsequent transplanting (replanting seedlings originated by seeds) (Sampaio et al. 2003). Rosewood doesn't require a specific substrate to germinate; Rosa and Ohashi (1999) didn't find any differences between germination of seeds in sandy soil, loam soil or combination of rocky soil and loam soil. This is a positive aspect that makes viable nursery techniques.

Moreover, Rosa et al. (1997) proved that shading (50%), NPK fertilization and daily irrigation promote survival (90%) and maximize seedlings' height growth (30 cm per year) of rosewood in nurseries. Santana and Barros (1997) propose mycorrhizal inoculum (a soil amendment which facilitates the return of native mycorrhizal fungi to depleted sites and is particularly effective on shallow or nutrient poor soils) to accelerate seedlings' growth in nurseries.

Rosewood can also be propagated by cutting (Vieira 1972). A study conducted by Sampaio (1987) showed that rosewood's stake harvested by young branches rooted 70% on average even without treatment. This technique offers great possibilities of high quality material selection for experimental plantations.

Rosewood plantings under partial shade (50%) of growth forest indicate a possibility of cultivating the species in agroforestry systems and forest clearing (Sampaio et al. 2003, Rosa et al. 1977). Planting in logging gaps is also an alternative to use and preserve other endangered species, such as *Swietenia macrophylla* King, commonly called mahogany (Lopes 2000).

Rosewood grows in red-yellow latosol, sandy soils and clay soils, exclusively in solid ground. These features are considered for species' cultivation but also it's possible to develop genetic improvement to select origins or progenies from oil greater productivity. It makes *ex situ* cultivation more attractive (Sampaio et al. 2000).

Tissue culture is also an alternative to reproduce greater genotypes of rosewood. Tissue culture (TC) is the cultivation of plant cells, tissues, or organs on specially formulated nutrient media (Cestari 1975). Under the right conditions, an entire plant can be regenerated from a single cell. Therefore, different plant parts such as plant gems, apical meristems, embryos, stem segments and root tips can be cultivated *in vitro* in nutrient media and aseptic environment (Grattaplagia & Machado 1998).

Cultivation *in vitro* of embryos and gems seedlings of rosewood were researched by Handa et al. (2005), the gems were obtained from seedlings sprouts cultivated at the nursery and the embryos were obtained from seeds in different stages of fruit development. It was observed 71% of survival and 53% of embryos' germination in pre-defined conditions.

National Institute for Amazonian Research develops studies about forestry of plantings that are homogeneous, combined and enriched with native forest species of Amazon region since the 1960s (INPA 2018). In 1998, the project Research & Development of products from rosewood leaves, financed by Amazon Bank (BASA) and coordinated by professor Lauro Barata, carried out 30 hectares experimental crops in Amazon with 10,000 rosewood trees and other aromatic plants focused on essential oil production from leaves.

Recently, Krainovic (2017) evaluated technical criteria for sustainable management of rosewood plantings that are scarce and barely inexistent. The research established standards of handling above-ground phytomass, validated sustainable management models in the use of sprouts through the description of performance of stump sprouts after harvest and sprouts management. The results indicated that it's possible to obtain rosewood essential oil from sustainable management. Undoubtedly, these research's results represent a great contribution for economic forestry and species' conservation.

It's clear that there is a need of technical and scientific knowledge concerning rosewood planting and development of sustainable management's planting techniques. Only then, it will be possible to reduce exploitation pressure about remaining rosewood's native populations.

3.3 EXTRACTION METHODS OF ROSEWOOD ESSENTIAL OIL

The extraction of essential oils in plants, as always, called a variety of industries attention: food industry, cosmetics industry, and perfumery. However, essential oils are usually isolated by different extraction methodologies depending on the plant, the type of an extract and the use of the essential oil (Bizzo et al. 2009).

As long as genus *Copaifera*, the oleoresin is stored in tubular cells presented in the trunk and branches. However, its spatial location in the trunk is imprecise and the oil can be found since the center to the tip of stem (Pinto et al. 2010).

In the species of this genus belonging to family Fabaceae, the oil comes from longitudinal axial secretory structures that are located in all parts of the tree (Pieri 2009). Various methods can be utilized for withdrawing oleoresin of copaiba. Unfortunately, most of the methods involve improper resources such as: cutting the tree trunk with axe, withdrawing the oil by mechanical pump and choking the tree with vines. All these practices are harmful leading to the plant's death (Veiga and Pinto 2002).

In the case of these oleoresins, the current referred method less harmful for the plant to obtain the oil is to drill the trunk by a manual hand drill (called by auger). This procedure should be done carefully to not kill the tree and must be done only with adult trees obeying time intervals (Moreira 2008, Pieri 2009).

Differently, the species of family Lauraceae, like *Aniba rosiodora,* are characterized by presence of parenchyma cells that produce oil. Secretory structures are related to oil and mucilage cells present, associated with axial parenchyma or ray parenchyma (Richter and Dallwitz 2019). These oil cells are surrounded by a cell wall (a structural layer situated outside the cell membrane) to avoid oil overflows and they are scattered throughout the tree (Richter 1981). That's why the extraction process of oil is done by cutting the trunk of the tree, branches and leaves (Barata 2001). In the following figures 10 and 11, it's possible to observe oil cells present in transverse section of trunk:

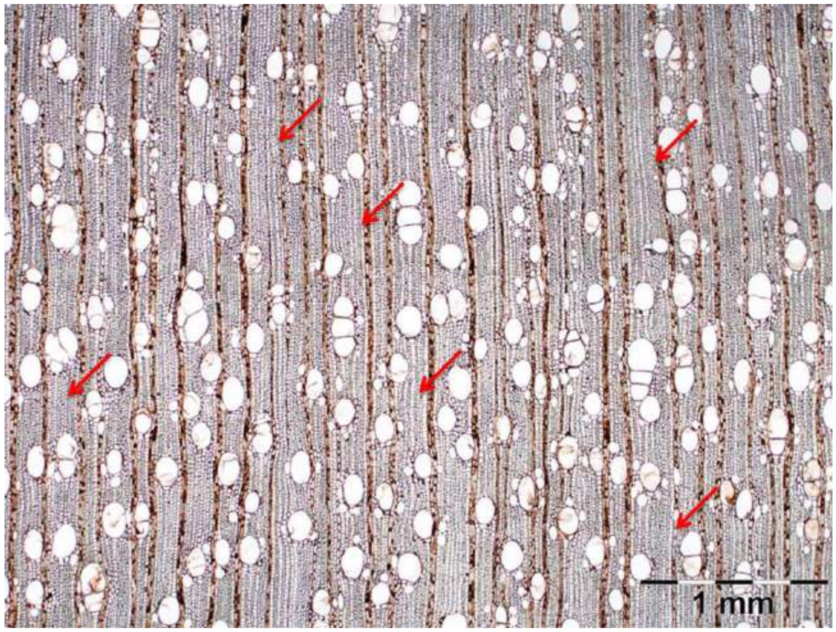


Figure 10: Transverse section of rosewood's wood. The oil cells are indicated by red arrows (Richter and Dallwitz 2019).

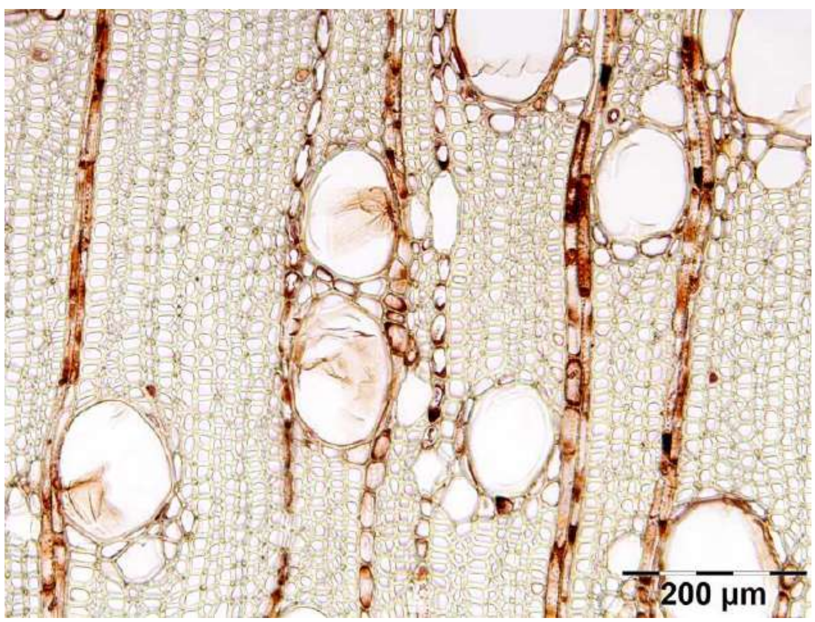


Figure 11: Transverse section enlarged of rosewood's wood showing oil cells (Ritcher and Dallwitz 2019).

Conventional techniques to obtain volatile oils involve distillation process, such as hydrodistillation and steam distillation. Although they present small variations, both processes are based on "the separation of the components of a mixture of two or more liquids by virtue of the difference in their vapor pressure" (Miall 1940).

In hydrodistillation, first, the plant material is packed into a still compartment, then water is added in sufficient amount (plant material may be completely immersed), and finally brought to boil. Hot water and steam act as the main influential factors to free bioactive compound of plant tissue. Indirect cooling by water condenses the vapor mixture of water and oil. Condensed mixture flows from the condenser to a separator, where oil separate from water by density difference (Simões et al. 2017, Aramrueang et al. 2019).

Steam distillation works essentially the same as traditional hydrodistillation, but the plant material doesn't get in touch with boiling water. The steam produced in a boiler is used as stripping gas to extract the oils. Steam is directed through the plant material. The mixture of hot vapors is collected and condensed in order to produce a liquid in which the oil and water form two distinct layers. The mixture oil-water can be separated by density difference (Simões et al. 2017; Irmak et al. 2008).

The extraction of rosewood's essential oil by steam distillation in Magaldi family distillery in the city of Maués in Amazon will be described:

The method has been used since 1920s when predation has started and it's still used nowadays. Carlos Magaldi family has manufactured the oil since 1950s in a plant inside the farm, the equipment used is simple but powerful to extract rosewood (FIORAVANTI, Carlos. O perfume da Amazônia. Fapesp.br. Accessed August 10th, 2020. <https://revistapesquisa.fapesp.br/o-perfume-da-amazonia/>).

The plant is working for third generation in Magaldi family and it produces annually around three thousand kilos of distillate oil. The production is earmarked for international perfume industry, especially for the United States, Europe and Japan. In addition, the plant covers an area of 15 hectares, splited in 11 squares, representing 25 thousand trees (MELQUIADES, Conceição. Especial Maués – Parte 2. Amazônia Press. <https://amazoniapress.com.br/especial-maues-parte-2/).

According to Carlos Magaldi, the plant has been working with the same equipment and methods since 1950s, the time that it was inaugurated. The production process works like this: using river water, the boiler generates vapor that makes a circular saw cutting trees' trunks. After, a mill triturates leaves, logs and branches. The ground product is taken to alembics (large metal cylinders) by conveyor and under each cylinder, water vapor is injected.

As far as the vapor is directed through the ground plant material, the oil is extracted and the vapor takes the oil out of the alembic. After that, rosewood's oil goes through a separation process from water and, finally, it is filtered. Figure 12 describes the production process in Magaldi's plant:

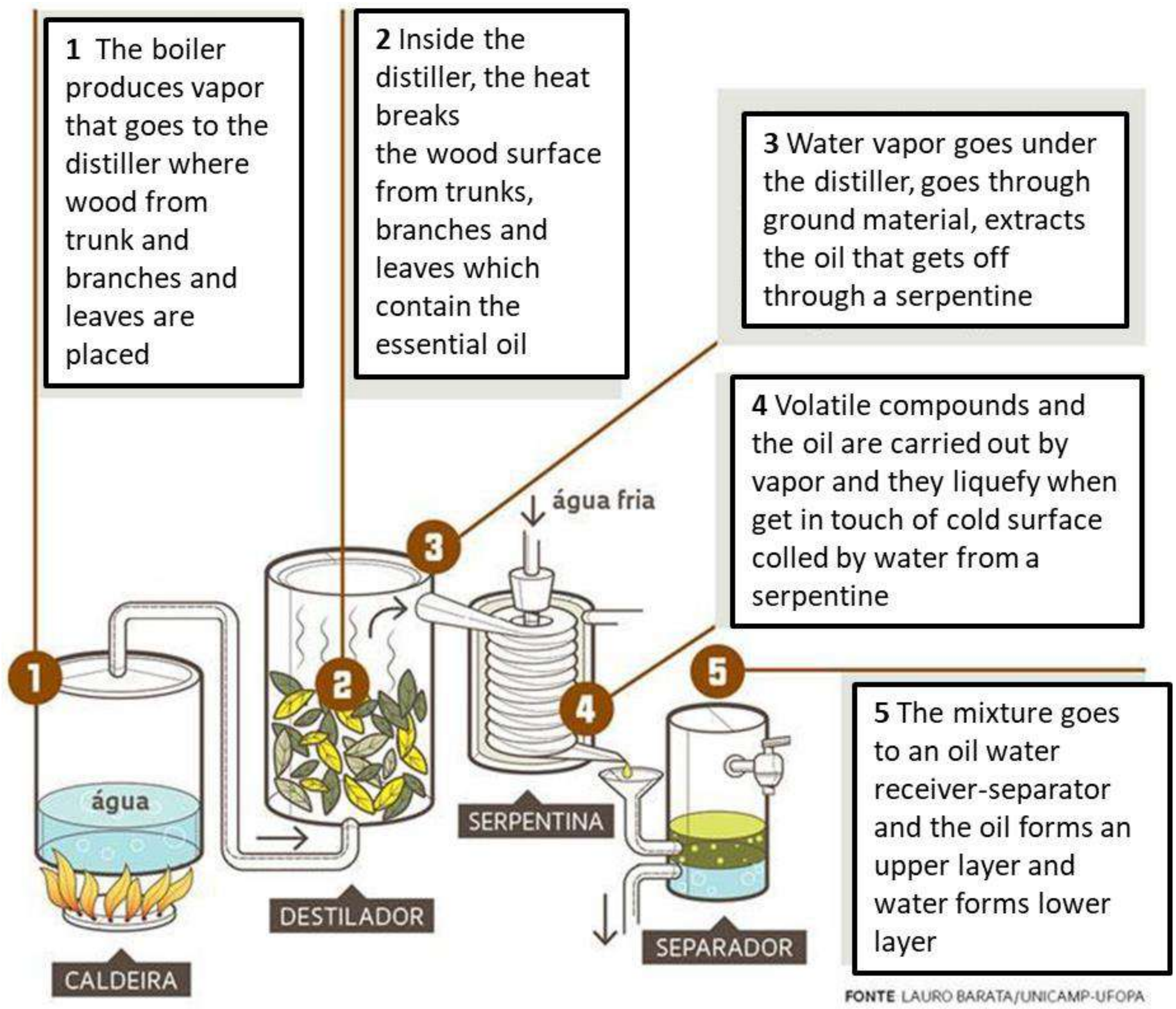


Figure 12: Infographic representing distillation process of rosewood's oil by steam distillation (with alterations from original) (Lauro Barata/Unicamp-UFOPA).

The photos below detail planting, rosewood's management and production process of oil in Magaldi family plant according to figures 13, 14 and 15:

Figure 13: Oil production: (1) supplying for boiler, (2) the engine that feeds the crusher, (3) other equipment from plant (FIORAVANTI, Carlos. O perfume da Amazônia. Fapesp.br. <https://revistapesquisa.fapesp.br/o-perfume-da-amazonia/>).

Figure 14: (1) Planting of rosewood tree; (2) branches and leaves being transported; (3) rosewood trunks separated to oil production and (4) equipment in the plant (Carlos Magaldi).

Figure 15: (1) Hundreds of rosewood seedlings in nursery; (2, 3, 4) planting and management of rosewood (Carlos Magaldi).

Described technology is relatively simple and aims, especially, to extract oil from wood, branches and leaves.

Nowadays, in Brazil, Brazilian Institute of Geography and Statistics (IBGE) monitors extractive production. The research Production of Plant Extraction and Forestry (PEVS) researches information about the amount and the value of production due to exploitation process of native plant resources and grown forest regions. However, PEVS doesn't refer to rosewood in their categories.

This indicates that there's no information about exploitation production of rosewood, forestry production, production value and existing and harvested areas of forest crops in IBGE platform. In order to relevance of species *Aniba rosiodora*, it would be worthwhile to include it in tracked categories by government.

1) Rosewood oil yield

The following description of essential oil yield calculations is based on Technical Communiqué 99 of the Ministry of Agriculture, Livestock and Food Supply published by Santos et al. (2004):

The yield of essential oil extracted from plant biomass can be calculated based on dry biomass or moisture free basis (MFB) and wet biomass or wet basis (WB). The method that uses MFB is standardized, while WB method is imprecise due to not considering the true amount of dry biomass used.

In calculating the essential oil extraction yield, it is necessary to know the moisture content of dry biomass, determined in accordance with Santos et al. (1998) and, for this purpose, the following equation 1 is used by MFB method:

$$C_O = \frac{V_O}{AGB - (\frac{AGB \ x \ U}{100})} \ x \ 100$$

Where, C_o is oil content (mL of essential oil in 100g of dry biomass) or extraction yield (%), V_O is extracted volume (mL), AGB is the aboveground plant biomass (leaves and twigs) measure in grams, $\frac{AGB \ x \ U}{100}$ is the amount of moisture or water present in the biomass and $AGB - \left(\frac{AGB \ x \ U}{100}\right)$ is the amount of dry biomass, water free or moisture free and 100 is the conversion factor to percentage.

This equation is widely applied in the determination of essential oil content in MFB, being that calculated value expressed as a percentage, which corresponds to volume/weight (mL of essential oil per 100g of dry biomass) and indicates the correct value of oil content in dry biomass.

The essential oil content can also be calculated in wet basis (WB), through the following equation 2:

$$C_O = \frac{V_O}{AGB} \ x \ 100$$

Where, C_o is oil content (mL of essential oil in 100g of wet biomass), V_O is essential oil volume, AGB is plant biomass and 100 is the conversion factor to percentage.

Equations 1 and 2 can be used to calculate essential oil yields in general, as well as to calculate rosewood oil yield.

In general, yield of *Aniba* oil ranges from 0.7% – 1.2% depending on plant material (trunk, branches, leaves) and collection area (Santana et al. 1997). Wood oil yield can ranges from 1% – 1.2% (May and Barata 2004).

In the city of Maués, local producers distinguish three types of rosewood that vary according to wood oil yield: “tucuribá” (higher oil yield, 15 liters/ton), “imbaúba” (10 liters/ton) and “cheirosa” (less than 10 liters/ton) (Leite et al. 2001).

Rosewood oil, traditionally, is made from the wood of the rosewood tree. However, since 1998, it has developed a project to obtain oil from leaves by Professor Lauro Barata. The project aims to preserve the live tree and ensure supplying of raw material to sustainable wood industry. This project was based on Otto Gottlieb researches (Gottlieb et al. 1957, Gottlieb et al. 1981) that showed the possibility to obtain rosewood oil from leaves by steam distillation.

Besides the leaf, it’s also possible to obtain oil from branches. The viability of oil extraction from leaves and branches is real, simply compare the require amount of plant material (leaves, branches, trunk) to produce, in liters, essential oil. One ton of youth leaves and branches produce around 24 liters of essential oil, meanwhile one ton of wood produces just 9 to 12 liters of oil (Alencar and Fernandes 1978). Such information definitely boosts new researches about alternative methods to obtain rosewood oil.

In 1971, Araújo et al. studies evaluated rosewood oil yield from leaves and twigs. The results showed that leaves’ oil yield (2.4%) was higher than twigs’ oil yield (1.1%). Moreover, one of the factors that caused variety in oil yield was the season when the leaves and twigs were collected. During rainy season (precipitable water over 250 mm), oil yield was lower than leaves’ and twigs’ oil yield collected during dry season. Araújo et al. (1971) has explained that lower yield is due to water circulation, relatively quickly, through oil cells. Once the elimination of oil’s constituent from cell requires a prior solubilization in water, it was concluded that linalool is eliminated during rain because it’s more soluble in water than oxides and other terpenes.

When it comes to collection season, Lima et al. (2007) checked leaves’ oil yield in two different periods of time. Collected leaves during season with lower precipitable water produced 2.3% oil yield meanwhile collected leaves during rainy season produced 2.1% oil yield. Despite a difference of less than 5%, studies agree with previous Araújo et al. (1971) studies.

On the other hand, Maia et al. (2007) observed another seasonal trend due to oil yield of rosewood leaves. The highest oil yield was verified during rainy season (2.2%) while the lowest oil yield (1.6%) occurred during September and October (lowest rate of precipitation). Authors believe that higher rate of precipitation in oil cells makes it easy the oil extraction process (hydrodistillation) to separate linalool.

In addition, Chaar's research (2000) evaluated oil yield from branches and leaves and also identified oil yield variations due to months of the year when the plant material was collected.

The plant material collection area is a factor influencing oil yield. Amazonas (2012) verified a difference of 1.22% between oil extracted from branches and leaves of Tapajós National Forest. Oil yield is variable even between rosewood trees from the same population, where they verified a difference of 1.92% between oil extracted from two individuals.

Schmaedeck (2012) studies also confirmed the effect of collection area. It was evaluated the oil yield of leaves collected in two different populations of *Aniba rosiodora*: Adolpho Ducke Forest Reserve and Maués State Forest. The average value of oil yield was 1.58% and 1.47% for Adolpho Ducke Forest Reserve and Maués State Forest, respectively.

Rosewood oil yield is one of the criteria that can be rated in *Aniba rosiodora* product. However, it is worth noting that yield itself is not the most important factor to be considered when the product of this species is evaluated. Fine perfumery industry around the world focuses mainly in linalool, chemical compound presents in rosewood oil. Linalool is the major constituent of oil and it's attractive for industry because it's a fixative (ingredient which prolongs the retention of fragrance on skin) and it comes with a bouquet of fragrances. If the oil yield is high and linalool content is low, the oil will have less commercial value for industry (Krainovic 2017).

The oil yield values of rosewood found in literature are organized in the table chart 1:

Bibliographic references	**Yield values according to plant material**		
	Leaves	Branches	Trunk
Araújo et al. 1971	1,4 – 2,6 %	1 – 2 %	-
Chaar 2000	1,2 - 1,5 %	1,3 %	-
Leite et al. 2001	2,4 %	2,4 %	1,1 %
May and Barata 2004	-	-	0,7 – 1,2 %
Maia et al. 2007	1,6 – 2,2 %	-	-
Lima et al. 2007	2,1 - 2,3 %	-	-
IBAMA Ordinance nº 9 of 25/08/11	1,9 %	1,1 – 1,9 %	1,1 %
Amazonas 2012	0,83 – 2,05 %	-	-
Schmaedeck 2012	1,43 – 1,57 %	1,66 %	-

Table chart 1: Rosewood oil yield values found in literature.

2) Chemical composition of rosewood oil from wood

In general, essential oils are natural volatile fractions, extracted from aromatic plants that that evaporate to room temperature. All these volatile chemical substances are formed by class of fatty acid esters, mono, sesquiterpenes, phenylpropane, aldehydes and aliphatic hydrocarbons (Santos et al. 2004).

The main volatile compound found in rosewood oil is linalool. Linalool (3,2-dimethyl-1,6-octadien-3-ol), chemical formula $C_{10}H_{18}O$, is a monoterpenic unsatured alcohol that presents pale yellowish color. Chemical structure of linalool can be visualized in figure 16:

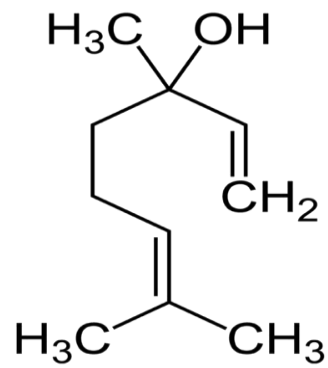


Figure 16: Chemical structure of linalool. (YMC. The sweet flavour of chiral separations. The Analytical Scientist. Accessed December 8th, 2020. <https://theanalyticalscientist.com/app-notes/the-sweet-flavour-of-chiral-separations-1>).

Linalool has an asymmetric carbon atom and can therefore exist as the optically active forms: S-(-) and R-(+). Linalool composition is a mixture of two optically forms: S-(+)-linalool, levorotatory enantiomer, called coriandrol and R-(-)-linalool, dextrorotatory enantiomer, called licareol. In Figure 17, we can see the chemical structures of the two stereoisomers:

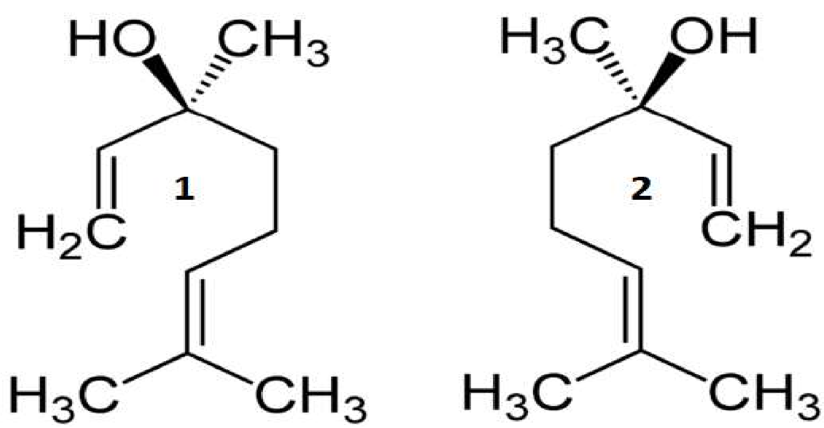


Figure 17: Chemical structures of (1) coriandrol e (2) licareol. (YMC. The sweet flavour of chiral separations. The Analytical Scientist. Accessed December 8th, 2020. <https://theanalyticalscientist.com/app-notes/the-sweet-flavour-of-chiral-separations-1>).

Linalool stereoisomers present distinct odor properties. The (-)-enantiomer was described to present woody, flowery, lavender-like, fresh notes, whereas the (+)-enantiomer elicited sweet, citric and herbaceous impressions. The different odor of enantiomers was also identified in oil derived from wood and leaf sources (Zellner et al. 2006).

Besides being the main compound in rosewood oil, linalool has been widely used as a common starting point for several syntheses, such as of linalyl acetate (Wei & Yuan 1997) and tested as bactericide and fungicide (Blaiche et al. 1995). In medical research, linalool has been implemented as sedative (Elisabetsky et al. 1995) and it has anticonvulsant properties (Elisabetsky et al. 1999). Linalool has a wide application thus its production in increasing quantity is necessary.

3) Differences and similarities in chemical composition of oil derived from trunk, branches and leaves

Linalool is identified as main constituent of oil derived from individuals of species *Aniba rosiodora,* independent of the plant material that the oil was extracted.

Comparing oil derived from leaves and trunk, a similar percentage of linalool was identified, being 81.45% of leaf oil and 85% of trunk oil (Zellner et al. 2006). The same research evaluated chemical composition of two types of oil trough gas chromatography – mass spectrometry (GC/MS). The chemical analysis confirmed similarity between the chemical profiles of the essential oils.

Some differences were verified. Oil derived from leaves is characterized by higher content of oxygenated sesquiterpenes comparing to oil derived from wood trunk that is composed with a slightly higher amount of oxygenated monoterpenes (Zellner et al. 2006).

Terpenes are largely found as constituents of essential oils. They are mostly hydro-carbons. Terpenes are linear or cyclic compounds composed of isoprene units which can be saturated or unsaturated, and modified in various ways (Felipe and Bicas 2017). The building block is a five-carbon isoprene (C_5H_8), which is presented in figure 18:

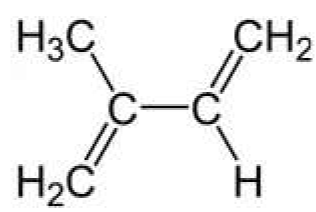


Figure 18: Chemical structure of isoprene – isoprene unit. (ROCHA, Jennifer. Terpenos. Presença dos alcadienos no cotidiano: os terpenos. Brasil Escola. Accessed December 16th, 2020. <https://brasilescola.uol.com.br/quimica/terpenos.htm>.

Monoterpenes and sesquiterpenes differ by quantity of isoprene unit in their structures. Monoterpenes present 2 isoprene units and 10 carbon atoms while sesquiterpenes present 3 isoprene units and 15 carbon atoms (Felipe and Bicas 2017). Among 37 identified compounds in oil derived from trunk and leaves in Zellner et al. (2006)

research, many belonged to terpenes class, such as: limonene and α-terpineol (monoterpenes), α-selinene and α-humulene (sesquiterpenes). Figure 19 presents the chemical structures of these compounds:

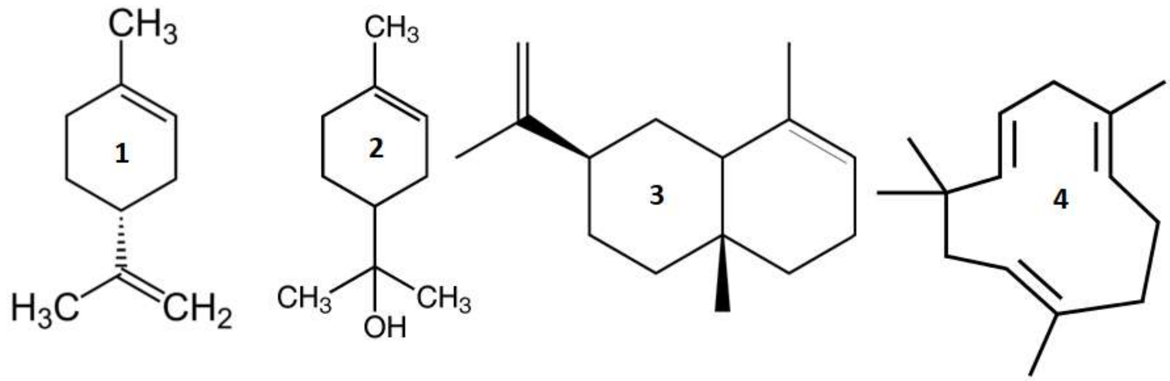


Figure 19: Chemical structure of (1) limonene, (2) α-terpineol, (3) α-selinene e (4) α-humulene. (McCord Research. Accessed December 16th, 2020. <https://mccordresearch.com.au/library>).

Whereas the most commonly used terpenes in perfume industry are oxygenated monoterpenes (Sell 1999), oil derived from trunk proved to be better in that way. In the other hand, oil derived from leaves presented a greater content of oxygenated sesquiterpenes and non-oxygenated (Zellner et al. 2006). These compounds are less volatile and, hence, they are used as fixative in perfumes (Curtis and Williams 2011).

Zellner et al. (2006) also performed gas-chromatography analyses to enable the determination of the racemic distribution of linalool in each sample, revealing a ratio of: 38.3% of R-(-)-linalool to 61.7% of S-(+)-linalool in the wood oil against 29.3% to 70.7% in the leaf oil, respectively. These results demonstrated the presence predominantly of coriandrol in oil extracted from leaves. However, there is a preference of perfume industry for the enantiomer licareol because of the fragrance. Therefore, considering the racemic distribution in these oils, we may conclude that oil production from leaves would be commercially interesting if the resulting product was predominantly composed of licareol.

Chemical profile evaluation in Zellner et al. (2006) research of rosewood oil obtained from leaves and trunk confirmed that the use of sustainable harvested rosewood leaves, rather than wood, in the extraction of natural linalool may represent a reasonable guarantee of long-term raw material source. Moreover, it can also be considered to use branches as plant material to extract oil.

The literature about chemical profile of rosewood essential oil derived from leaves is still scarce. So, it's important to know about chemical profile of leaf oil in more detail.

Fidelis et al. (2012) characterized essential oil derived from leaves collected from 4-year-old trees and as a result, verified that chemical profiles from wood and leaf oils are similar. It is important to point out that the oil analyzed has fragrance characteristics similar to *A. rosiodora* wood oil. This study showed that rosewood plantations in the Amazon could be the answer to the conservation of the *A. rosiodora* species. Thus, from an economic point of view, it seems that young plants can produce essential oil with the quality required by the industry in a reasonable yield (0.75%).

To verify the viability in oil production from leaves of young plants, Fidelis et al. (2013) characterized and distinguished essential oil extracted from the leaves collected from different ages (4, 10 and 20 years old). Chemical analysis comparing essential oil of these trees showed that chemical composition are similar, but some compounds weren't identified in samples of 10 and 20-year-old tress according to the table chart 2 below:

	Age of trees		
Compounds	**4 years old**	**10 years old**	**20 years old**
(1) Fenchol	X	X	N.I.
(2) 1-Terpinenol	X	N.I.	X
(3) Trans-Dihydrocarvone	X	X	N.I.
(4) ß-Citronellol	X	X	N.I.
(5) α-Cubebene	X	X	N.I.
(6) ß-Guaiene	X	N.I.	X
(7) ß-Chamigrene	X	N.I.	X

X*: presente compound, N.I.*: not identified.

Table chart 2: Not identified compounds in samples of essential oil from leaves collected from different ages trees. (Fidelis et al. 2013).

Chemical structure of these compounds can be seen in figure 20:

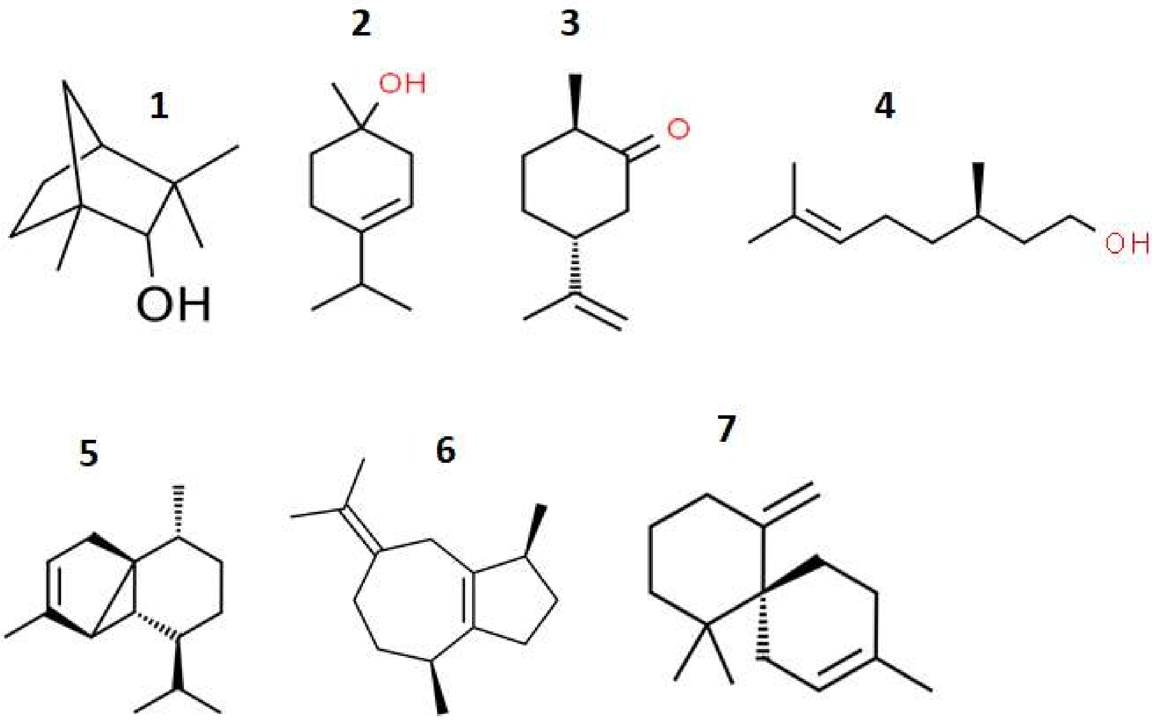


Figure 20: Chemical structure of not identified compounds in samples of oil derived from leaves of trees with different ages: (1) Fenchol, (2) 1-Terpinenol, (3) Trans-Dihydrocarvone, (4) ß-Citronellol, (5) α-Cubebene, (6) ß-Guaiene e (7) ß-Chamigrene. (McCord Research. Accessed December 17th, 2020. <https://mccordresearch.com.au/library>).

Most of the compounds were identified in samples of oil from leaves of all the trees and linalool percentage found in samples was similar, as 83.2% for 4-years-old tree; 90.5% for 10-years-old tree and 87.1% for 20-years-old tree (Fidelis et al. 2013). Thus, from an economic point of view, it seems that young plants from four years-old can produce essential oil with quality similar to those from older trees.

In addition, rosewood oil can be also produced from sprouts of branches and leaves. Takeda (2008) evaluated linalool content in 3 and 5-years-old plantings. Average values can be found in table chart 3 below:

	% Linalool	
	3-years-old plantings	5-years-old plantings
Leaves	49.78	38.10
Branches	63.83	65.12

Table chart 3: Average values of linalool content in essential oil obtained from sprouts of branches and leaves of 3 and 5-years-old rosewood plantings (Takeda, 2008).

In general, average values of linalool content don't present great variation in the different ages analyzed. Furthermore, the highest concentration of linalool was found in oil derived from branches.

Higher values of linalool in leaf oil and breaches oil were found in Chaar's (2000) studies: 78% of linalool in branches oil and 68% of linalool in leaf oil. Takeda (2008) and Chaar (2000) showed results with a higher concentration of linalool in oil branch than in oil leaf. Linalool content in leaf oil and branch oil in these studies proved the viability to extract oil from these plant organs.

4) Sustainability in rosewood oil production

The presence of linalool in oil extracted from leaves and branches allowed a new perspective related to rosewood essential oil production. The extraction of oil from leaves and branches shows up as an alternative to avoid cutting down trees (May and Barata 2004).

For Santana et al. (1997), the extraction of oil from leaves is a potential industrial activity and this trend can be confirmed by exportation of 2,000 kg of rosewood leaves' oil in the year 2010-2011 by the company Benchimol and Irmão located in Manaus, average selling price was US$ 183/kg (Amazonas 2012).

In addition, the management of rosewood plantations can also turn oil extraction as a sustainable practice. Studies have already shown the viability to commercially explore cultivated trees, avoiding the deforestation of native trees. It is known that planted rosewood trees produce essential oil in somehow different from oil extracted from trees found in nature, although studies already carried out suggest that the cut of young individuals of planted rosewood with a maximum of five years of age allows oil production from leaves and branches in the industrial sector (Barata 2011, Fidelis et al. 2012).

Another sustainable alternative is the use of technique of treetop pruning in management of rosewood plantation for biomass production (leaves, branches). The efficiency of treetop pruning for biomass production of sprouts is highlighted in Leite et al. (2011) research. In this study, rosewood presented regrowth power satisfactory, producing secondary branches and sprouts with the tree top pruning in 1 or 2-years-old trees.

Periodic pruning generates plant reinvigoration, increase the production of plant mass and consequently, increase the volume of leaves and branches. Leaves and branches become thicker and it contributes to obtain a greater volume of oil extracted (Chaar 2000). Oil production is directly proportional to aboveground biomass (Sampaio et al. 2005), hence, Takeda (2008) evaluated different types of treatments (varying the percentage of pruning and fertilization) to produce greater amount of oil. In conclusion, the 100% pruning treatment and no fertilizer produced more oil as it stimulated greater production of biomass from sprouts of branches and leaves (16 ton/ha). In addition to proving the efficiency of tree pruning, the results showed that there is no need for investments in fertilization for rosewood planting in order to obtain more oil volume.

In addition to preserve species *Aniba rosiodora,* periodic pruning system is a forestry technique that allows the production of more vigorous sprouts by the plant, greater volume of biomass and in less time. These features ensure a faster financial return and the production process is less harmful to the plant (Takeda 2008).

The results of these investigations have been promising as they show the possibility of carrying out successive pruning of cultivated individuals without affecting the tree’s vitality, stimulating greater biomass production and, consequently, oil production. However, despite being a low capital production system and commercially interesting, it is still necessary to develop studies on the management of monocultures (in the case, rosewood) to produce biomass (leaves and branches) as there is still no published information about pests and diseases that can limit the production system (Sampaio et al. 2005).

3.4 BACKGROUND OF ROSEWOOD OIL INCLUSION IN THE APPENDIX II OF CITES

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), signed by Brazil in 1975, is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plant does not threaten the survival of the species. CITES provides a certified system and licenses to control trade in endangered species with standards that apply only to international transactions (CITES 2021).

To this end, it attributes to producing and consuming countries their share of the common responsibility and establishes the necessary mechanisms to guarantee the non-harmful exploitation of populations. Over 38,7000 species are protected by CITES against over exploitation trough international trade. They are listed in the three CITES Appendices. The species are grouped in the Appendices according to how threatened they are by international trade (CITES 2021).

The species *Aniba rosiodora* was included in Appendix II of CITES in 2001. Appendix II lists species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled. It also includes so called "look-alike species", i.e., species whose specimens in trade look like those of species listed for conservation reasons. International trade in specimens of Appendix II species may be authorized by granting of an export permit or re-export certificate (https://cites.org/eng/app/index.php).

The inclusion of rosewood in Appendix II of CITES is a consequence of the intense exploitation of native trees for oil production. The production of essential oil by steam distillation has taken place since the 1920s and countries as Peru, Colombia, Guyana, Suriname and French Guiana were rosewood oil exporters in the past, but nowadays Brazil is the only world exporter (CITES 2010d).

Natural populations have seriously declined due to oil extraction that occurred in the past and the few remaining individuals are found in remote areas with significant signs of lack of regeneration (May and Barata 2004).

In Brazil, since the beginning of the last century, the essential oil has been used unsustainably and through the historical data of extraction of the species, a population reduction of 46% is estimated. Access to natural rosewood subpopulations has become increasingly difficult and we can't forget the continuous decline in its extent of occurrence and the quality of its habitat due to logging and extractive activities.

In Colombia, the Ministry of Environment and Sustainable Development declared the species *Aniba rosiodora* as an endangered species in the national territory through

Resolution 192 of 2014. Colombian populations of rosewood are extremely scarce and the existing ones are represented by few individuals in groups. Unfortunately, the use of rosewood in past years has led to local extinction in most areas of the Colombian Amazon. In the Amazon region of Colombia, in the village of Tarapacá, there was a rosewood exploration unit for oil extraction that operated for many years, but nowadays, there is nothing working, only abandoned machinery can be found in the place.

In Peru, the species *Aniba rosiodora* is categorized as vulnerable and included in the Red List of Endangered Species of Wild Fauna and Flora, prepared by IUCN (Supreme Decree nº 043-2006-AG). There is no data on the wild population, but it is inferred that it is greatly reduced due to historical overexploitation, establishment of monocultures in their habitat and deforestation resulting from the extraction of timber species. The inclusion of species on the red list indicates that populations are at a level of sensitivity that can put them in danger if alternatives for their conservation are not adopted (Ministerio del Ambiente Perú 2015).

In view of the world situation, CITES established at the fifteenth meeting of the plant committee (CoP15) some resolutions to the States in the commercialization area and the rosewood importing parties that work with the plant committee. Among them, identify the best methods or potential methods for identifying the essential oil extracted from rosewood and even from the wood. Therefore, there is an international effort to help identify specimens of the species and preserve the populations that still exist.

3.5 PRODUCT QUALITY OF THE SPECIES *Aniba rosiodora*

Rosewood essential oil is a valuable product known for its sweet and woody aroma, besides its economic value for the perfume industry. Currently, rosewood essential oil is produced on a small scale in the state of Amazonas and it was sold as a price of US$ 233.66/kg in 2016 (MDIC 2016).

Essential oil extraction from leaves and branches developed in recent years and previously mentioned in this work, have promoted investments by traditional indigenous communities and business sectors in rosewood plantations. Two main points mark this new phase on rosewood essential oil production: (1) cultivation as a substitute for extraction and (2) adoption of more sustainable management techniques (Laura et al. 2018).

Despite these advances, there are still some gaps to be filled due to rosewood production chain. Amongst them, forestry-related problems, no quality genetic material selected for seed production and planting programs. These are recurrent problems in Amazonian aromatic essential oil production, which results in difficulties in standardizing the quality of the final product, what is required by perfume and cosmetics industry. For these reasons, Lara et al. (2018) cited 3 crucial steps for rosewood trees cultivation: (1) study of chemical variability in remaining natural populations, (2) knowledge of oil properties that are most interesting for industry and (3) development of genetic improvement programs to improve production techniques. In fact, these points are relevant when considering planting of trees and production of final product: essential oil.

Regarding the final product, one of the most notable features of rosewood essential oil is the presence of linalool as the main compound, which provides a fragrance with a unique aroma. Production and commercialization of linalool directly linked to perfume and cosmetics industry.

Linalool isomers proportion (licareol and coriandrol) in rosewood essential oil also directly affects the use of the product by industry. That's because linalool isomers have different fragrance profiles (Sugawara et al. 2000). Coriandrol is perceived as sweet, floral, herbaceous and petitgrain like with citrus and fruity notes while licareol has a woody, lavender-like aroma (Aprotosoaie et al. 2014).

Moreover, oil obtained from leaves and branches, such as trunk oil, although they present a high concentration of linalool, they may show high variability in their enantiomeric distribution. Knowing about this variability may increase commercial interest in oil extracted from leaves and branches (Almeida et al. 2009). Thus, one of the ways to evaluate rosewood final product is assess enantiomers presence, once it directly alters oil aroma.

Laura et al. (2018) evaluated differences and similarities in relation to linalool enantiomeric distribution of leaf-oil and branch-oil of native Amazon trees from two populations: Adolpho Ducke Forest Reserve and Maués State Forest. Linalool enantiomeric distribution was analyzed by chiral gas chromatography. Variation in linalool enantiomeric distribution was high between analyzed samples. Table chart 4 describes the biggest and smallest proportion between the enantiomers 3R-(-)-linalool (licareol) and 3S-(+)-linalool (coriandrol) found in samples:

Sample	Proportion licareol:coriandrol
Branches collected in Adolpho Ducke Forest Reserve	44.2:55.8
Leaves collected in Maués State Forest	5.2:94.8

Table chart 4: Enantiomers proportion licareol:coriandrol in branches and leaves collected in Adolpho Ducke Forest Reserve and Maués State Forest (Lara et al. 2018).

Despite this variation, all samples presented enantiomer 3S-(+)-linalool as main compound. It is possible that oil extracted from different plant organs presents different olfactory qualities, which suggests that selective extraction of leaves and branches can be useful in specific development of perfumes by industry.

Santana et al. (1997) also reported a proportion between 3R-(-)-linalool and 3S-(-)-linalool of 22.7:77.8 in oil derived from leaves of rosewood trees from Curua-Una (state of Pará), which indicates that coriandrol was the enantiomer found in greater proportion. The authors attribute this variability to genetic characteristics of individuals, emphasizing that these differences can significantly impact essential oil production due to olfactory qualities of enantiomers.

Zellner et al. (2006) analyzed linalool enantiomeric distribution in oil derived from wood and leaves and he obtained the following proportions in table chart 5:

Sample	Proporção licareol:coriandrol
Oil derived from wood	38.3:61.7
Oil derived from leaves	29.3:70.7

Table chart 5: Proportion between enantiomers licareol:coriandrol in oil derived from wood and leaves (Zellner et al. 2006).

Zellner et al. (2006) showed that the highest percentage observed among the enantiomers was 3R-(-)-linalool in wood compared to the percentage found in leaves. While in Lara et al. (2018) studies, it was observed a greater proportion of 3R-(-)-linalool in samples of oil derived from branches in comparison to leaves. Thus, the results suggest that linalool enantiomeric distribution of oil extracted from branches is more similar to enantiomeric distribution found in wood oil than in leaf oil.

However, Chantraine et al. (2009), when studying 82 rosewood trees collected from ten localities in French Guiana, observed a different trend in linalool enantiomeric distribution. Almost all the trunk wood samples of young or big trees showed a R-(-)-linalool/total linalool ratio of 100%. Two samples were the two exceptions which contained a slight proportion of S-(+)-linalool estimated at 5-10%. The samples from branches indicated a S-(+)-linalool/total linalool ratio ranging from 5% to 28%, showing a clear inverted trend regarding Lara et al. (2018) results.

Leaf samples analyzed by Chantraine et al. (2009) showed a high S-(+)-linalool/total linalool ratio from 78% to 89% similar to Lara et al. (2018) results. According to Terezo et al. (1972), the presence of a high amount of S-(+)-linalool in leaf oil is not appreciated by perfumers, which is an argument against a possible commercial exploitation of leaves.

However, in practice, this is not observed. Rosewood essential oil from leaves and branches has been produced annually by Carlos Magaldi family since 2011 in Maués, state of Amazonas and it is sold to international perfume companies. The plant has manufactured rosewood oil since the 1950s. Not long ago the product was all extracted from trunks and branches that came from managed areas forest. Nowadays, in the plant, leaves and dry twigs are also crushed and the material is used for oil manufacturing (Plantas da Amazônia são usadas na indústria de cosméticos e perfumes. Natureza. Accessed January 14th, 2021. <http://g1.globo.com/natureza/noticia/2014/01/plantas-da-amazonia-sao-usadas-na-industria-de-cosmeticos-e-perfumes.html>).

According to Santana et al. (1997), there would be greater commercial interest if a selection of germplasm could produce oil from leaves with a predominance of 3R-(-)-linalool with a woody aroma. Germplasm is living tissue from which new plants can be grown, e.g., it can be a seed or another plant part – a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant. Germplasm contains the information for a species' genetic makeup, a valuable natural resource of plant diversity (UNIVERSITY OF CALIFORNIA, DIVISION OF AGRICULTURE AND NATURAL RESOURCES. Germplasm. Ucdavis.edu. Accessed January 14th, 2021. <http://sbc.ucdavis.edu/About_US/Seed_Biotechnologies/Germplasm/>).

The similarity observed in the linalool enantiomers proportion between oil from leaves and wood (Lara et al. 2018) confirms the potential to replace the traditional oil production system by cutting trees to a successive pruning production using branches and leaves to produce essential oil. The replacement of the traditional tree cutting system to management through pruning system is justified by the maintenance of oil quality and reduction of environmental impacts in its production. In terms of production sustainability, the management has numerous benefits from an economic point of view and also for the conservation of the species *Aniba rosiodora*.

Considering these results, the variability in linalool enantiomers distribution is high compared to other aromatic plant oil containing linalool. Studies about species of genus *Ocimum basilicum* L. showed an approximate proportion of 100% of enantiomer R-(-)-linalool similar to the oil of *Cinnamomum camphora* of China, which is also used in perfume industry (Casabianca et al. 1998, Ravid et al. 1997). The exclusive presence of the enantiomer S-(+)-linalool is found in literature of oil extracted from species *Lippia alba* Mill, which can be an alternative to supply the market of essential oils containing linalool (Siani et al. 2003).

Despite research on the aromatic potential of other species containing linalool, rosewood is still overvalued for containing a unique fragrance that serves as a raw material for perfume industry, although it is currently available in small quantities.

International demand for rosewood essential oil has been constant, with untapped growth potential in the world market. Consequently, many producers are interested in the commercial production of rosewood oil. However, they find a lack of technical information on production management (May and Barata 2004) and lack of clarity in the standardization established by current legislation (Krainovic et al. 2018). Although the development of technical criteria for rosewood management is recommended by law (IBAMA Normative Instruction nº 09 of 08/25/2011), there is a lack of studies about the effectiveness of harvesting standards to optimize the oil extraction from rosewood under commercial production conditions and about the influence of specific harvesting patterns on oil yield and quality (Krainovic et al. 2018).

The rosewood essential oil production chain requires a complete understanding of management, from the proper management of trees to quality oil production for international market. It is necessary to look upon the extensive compositional variability of rosewood oil from managed plantations, as well as factors that may influence this variability. Although the oil consists mostly of low molecular weight monoterpenes and sesquiterpenes (Krainovic 2011), the range of smaller components gives the unique fragrance bouquet. Consequently, the conditions under which rosewood trees are grown are able to stimulate the redirection of metabolic pathways, resulting in the biosynthesis of different compounds (Morais 2009).

Krainovic et al. (2018) presented some hypotheses regarding the influence of biomass on the chemical composition of rosewood oil, including: (1) growing region of trees influences yield and chemical composition of essential oil, (2) the use of different parts of the plant for oil production will produce different yields and different chemical compositions of the final product, (3) the use of regenerated biomass will produce essential oils with different characteristics from oils produced from first-crop biomass in a sequential management system. Thus, to test these hypotheses, Krainovic et al. (2018) studied the effects of growing region on rosewood oil composition, characterized oil variation from different parts of the tree and investigated the feasibility of sequentially harvesting rosewood.

The samples were collected in Maués and Novo Aripuanã and differences in chemical composition of the essential oil were observed between two regions. The number of components found in the oil was different and some compounds were only present in samples from one region. Table chart 6 below describes the differential substances between the plantations for each part of the plant where the oils were extracted:

Part of the plant	Region	Number of compounds	Essential oil components that differentiate regions
Trunk	Maués	47	trans-nerolidol, viridiflorol, α-farnesene, aristolene epoxide, isoaromadendrene epoxide
	Novo Aripuanã	51	p-cimene, mircenol, β-citronelol, α-amorphene, β-trans-guaiene, cadinene, guaiol
Branches	Maués	49	eucalyptol, ledene oxide
	Novo Aripuanã	56	p-cymene, nerol acetate, gurjunene, α-amorphene, Y-celinene, β-trans-guaiene, aromadendrene dihydro
Leaves	Maués	53	camphene, eucalyptol, borneol, β-trans-guaiene, 3-methoxymethoxy 3,7,16,20 tetramethyl eneicosa
	Novo Aripuanã	60	benzaldehyde, linalyl acetate, trans-dihydrocarvone, α-cariofileno, α-humuleno, α-amorphene, y-celinene, aromadendrene dihydro, cadinene, ledol, aristolene epoxide

Table chart 6: Differential substances between plantations in Maués and Novo Aripuanã for each tree compartment from which the essential oils were extracted (Krainovic et al., 2018).

Regarding the essential oil yield extracted from different parts of the plant, yield values differed being the lowest found in branches oils (1.14%), a little higher in leaf oils (1.54%) and the highest yield in trunk oil (1.77%). This difference occurs regardless of growing region. Trunk and branch essential oil yield agree with the results reported by Chantraine et al. (2009) for rosewood grown in French Guiana.

The results obtained in the study by Krainovic et al. (2018) contradict the information in Brazilian legislation (Normative Instruction IBAMA nº9 of 08/25/2011) which affirms that rosewood leaves present the highest yield values. However, although the legislation is not clear in this regard, there are indications that the data contained in the legislation are based on natural rosewood plantations, hence, suggesting a need for updates to legal provisions so that parameters related to rosewood plantation trees are included (Krainovic et al. 2017).

The parts of the plant used for essential oil extraction have intrinsic differences and can produce different types of oil with unique characteristics (Krainovic et al. 2017). These features can influence chemical composition of the final product. Table chart 7 describes substances that differentiate the essential oils extracted from different parts of the plant:

Reference compartment	Not in wood oil	Not in branch oil	Not in leaf oil
In branch oil	α-pinene, linalyl acetate, nerol acetate, cyclosativene	-	Ciclosativeno
In leaf oil	α-pinene, ledene oxide	α-farnesene, α-bisabolol, isoaromadendrene epoxide	-

Table chart 7: Substances that differentiate the essential oils extracted from different parts of the plant (Krainovic et al., 2018).

α-Pinene, which is formed during the biosynthesis of limonene (Xu et al. 2017), is the most widespread pinene isomer and is highly desired by the flavors and fragrances industries (Mani et al. 2017). Oxidative metabolization of α-pinene results in other compounds (α-pinene oxide, campholene aldehyde, verbenone and verbenol) that are also important in the chemical and cosmetic industries (Cánepa et al. 2011). Due to their importance, mechanisms through which this terpenoid may be selectively produced, whether by enzymes or microbial transformation, are being studied (Paduch et al. 2016). Figure 21 presents chemical structure of α-pinene:

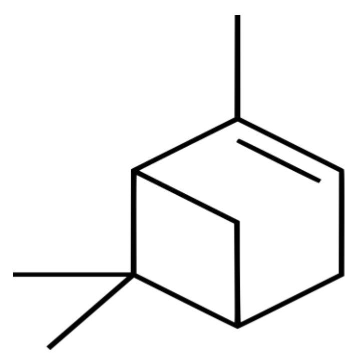


Figure 21: Chemical structure of α-pinene (McCord Research. Accessed January 25th, 2021. <https://mccordresearch.com.au/library>).

Oil chemical composition is also associated with harvest time and essential oils from first harvest and resprouting were compared. The differences between the oils extracted from plant material of first harvest and resprouting, regardless of growing region and part of the plant, were the presence of geraniol only in oils extracted from first harvest plant material and the presence of myrcenol only in oils from plant material resprouting. Table chart 8 lists the substances that differentiate first harvest and resprouting:

Compartment	Only present in first harvest	Only presente in resprouting
Branches	α-pinene, geraniol, cyclosativene	borneol, myrcenol
Leaves	ρ-cymene, geraniol, isoaromadendrene epoxide	myrcenol, gurjunese

Table chart 8: Substances that differentiate essential oils extracted from each plant part collected in harvest and resprouting, regardless of cultivation region (Krainovic et al. 2018).

In conclusion, essential oils of Central Amazonian from different locations, different plants parts and different harvesting times vary in yield and quality in relation to the present components. International industry with interests in rosewood oil is focused on its chemical composition, with emphasis on the presence of linalool and on the bouquet of fragrances of minor components. If essential oil yield is high, but the content of these substances is low, the oil will be less valuable to industry, resulting in a lower price (Krainovic et al. 2018).

The proportion of materials to be used in essential oil extraction should be chosen focused on adding value to the final product (e.g., branches have the lowest oil yield, but extracts contain valuable substances). Thus, Krainovic et al. (2018) has concluded that adding mixtures of branches and leaves to wood material during the preparation phase of essential oil extraction will confer the presence of certain substances in valuable proportions for commercially interests, which must be validated by olfactory characteristics.

Regardless of growing region, Krainovic et al. (2018) verified that there is a quality gradient between oil extracted from harvest and resprouting, thus, new product lines can be created to exploit these differences. Although the compounds and characteristics required by industries are present in the material from first and second harvests, their proportions differ. Thus, harvest cycle planning should be considered for these differences, as they can be used to strengthen the production chain for rosewood essential oil.

3.6 ADULTERATION IN ROSEWOOD OIL

Another concern of international industry that buys oil extracted from rosewood is oil adulteration. Essential oils are used all over the world, but adulteration issue can harm trade development (Juliani et al. 2006). Typically, essential oil prices range from a few to thousands of euros and vary from year to year. Prices correlate with the importance of essential oils use and they may be the result of adulteration for dishonest profits in some cases (König et al. 2004).

Essential oils adulteration can happen in a number of ways. In some cases, falsification can be done by adding cheaper synthetic material, cheap volatiles from other natural sources, or vegetable oils to add weight (König et al. 2004). Adulterations can also involve the partial or total replacement of original plant by other plants, or addition of non-volatile products (Salgueiro et al. 2010). All of these tampering methods can degrade quality and, by adding one or more synthetic compounds, tampering can lead to safety issues. Consequently, authentication is an important topic for consumer protection and for the quality of essential oil production (Mosandl 2004, Do et al. 2015).

In the case of rosewood essential oil, a common practice is to mix genuine rosewood oil with synthetic linalool, which is a cheaper compound synthesized in the laboratory. Therefore, there is a need to develop techniques to identify this type of adulteration in essential oil.

Souza et al. (2011) used direct infusion of samples via electrospray ionization mass spectrometry (ESI-MS) to characterize by fingerprint genuine samples of *Aniba rosiodora* essential oil. This technique has been used in other studies (Moller, Catharino and Eberlin 2007) and it has been proved to be versatile and fast and is applied with no pre-separation and with little or no sample preparation. ESI-MS by fingerprint was able to detect the presence of 10% of synthetic linalool in an adulterated oil sample.

In addition, PCA analysis was carried out to statistically analyze data, which places samples in three very well-defined groups: (1) pure wood oil, (2) pure leaf oil and (3) synthetic oil. Figure 22 below presents PCA analysis for samples of rosewood wood and leaf oils and synthetic linalool:

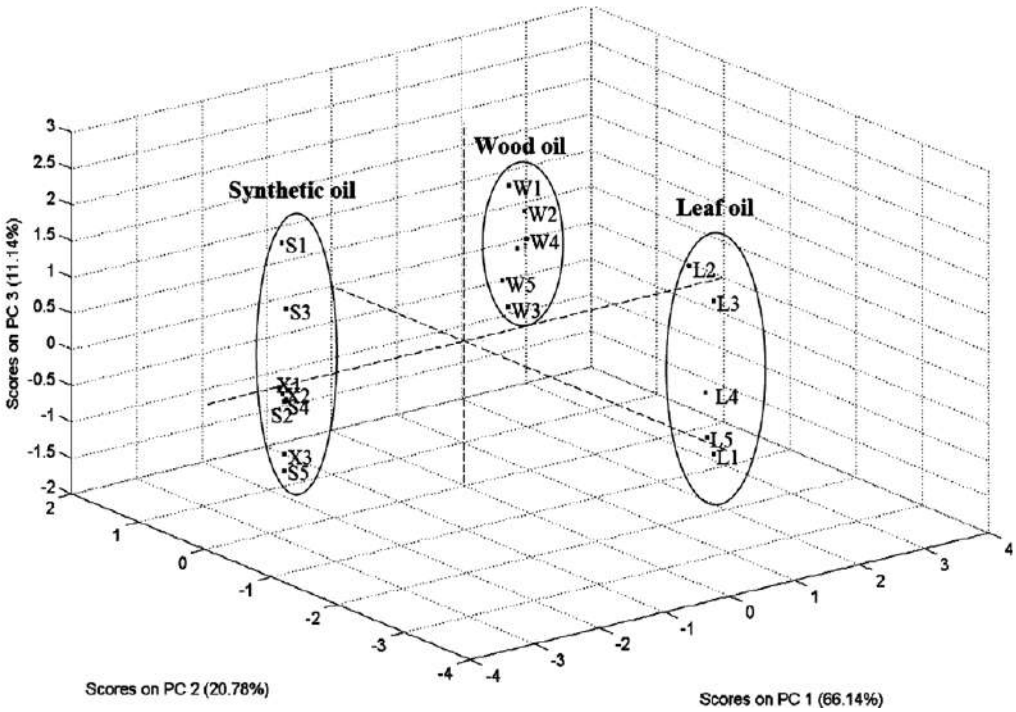


Figure 22: PCA analysis of the ESI-MS data for samples of rosewood wood and oils and synthetic linalool: wood (W1-W5), leaf (L1-L5), synthetic (S1-S5), and mixtures of wood and synthetic oils (X1-X3) (Souza et al. 2011).

Therefore, the technique used allowed the characterization and identification of the types of samples. This type of determination is commercially interesting, as especially fine perfume industry enriches natural linalool for its aroma and also its fixation property (Souza et al. 2011).

3.7 APPLICATION OF NIRS METHOD TO *Aniba rosiodora*

In recent decades, near infrared spectroscopy (NIRS) has been recognized as a fast, effective, non-destructive and cost-effective technique. NIR spectroscopy has been applied as a qualitative and quantitative tool in forest industry. Conventionally, NIR spectroscopy is sensitive enough to detect differences in chemical composition of trees growing in different locations (Schwanninger 2011) and to predict extractives yield of different wood producing forest species (Poke et al. 2006, Silva et al. 2013).

NIR spectroscopy has been shown to be efficient for characterization of several essential oils from plants, including oils of basil, chamomile and oregano (Steur et al. 2011, Wedding et al. 2008). It has also been used to determine the content of essential oil from fennel, cumin, dill and coriander, reliably and non-destructively (Schulz et al. 1998).

There were no studies proving that NIR spectroscopy could be used to estimate rosewood essential oil yield in wood samples until 2016. In that same year, Amusant et al. (2016) verified the feasibility of applying NIR spectroscopy for this purpose. In his studies, he used diffuse reflectance with a Fourier transform spectrometer on rosewood ground wood samples before the extraction process. NIR spectra of the wood powder samples were examined to investigate if there were specific spectral regions related to the essential oil yield variation. Differences were detected in absorption spectrum between the highest and lowest yield of essential oil from samples (0.6% and 3.7% respectively).

Then, a total of 319 samples were used to develop a calibration model to predict oil yield. The results suggested that the high level of accuracy attained from the model, both in cross-validation and independent validation, demonstrates the potential for developing models that could predict oil yield in rosewood wood (Amusant et al. 2016).

NIR spectroscopy proved to be an alternative to hydrodistillation (oil extraction process) by predicting oil yield of wood powder samples. Previously, it would only be possible to calculate oil yield after extraction process, which takes time and resources. Thus, it is important to develop methods that can allow trees selection with high oil yield potential before harvesting, or selection of high oil yield genotypes for elaboration of a sustainable rosewood management plan.

The high level of precision obtained with PLS model developed by Amusant et al. (2016) demonstrates the potential to develop other models that can be applied to powdered wood. However, it is noteworthy that NIR spectrum of wood was obtained in controlled laboratory conditions, also using a laboratory instrument. Therefore, application of portable NIR instruments that enable a quick, non-destructive analysis carried out in field should be explored.

NIR spectroscopy can not only be applied to predict essential oil yield, but it is also a technique used for identification/classification of forest species. Works by Forest Products Laboratory research group together with Laboratory of Automation, Chemometrics and Environmental Chemistry of University of Brasília evidenced the potential of this technology for discrimination of Amazonian woods, demonstrating its applicability in samples from different countries and also the use of portable spectrometer (Pastore et al. 2011, Braga et al. 2011, Bergo et al. 2016, Soares et al. 2017, Silva et al. 2018, Snel et al. 2018, Rocha et al. 2021).

In addition, NIRS technology was included as one of the techniques recommended by the guide to good practices for identifying wood for forensic purposes, published in 2016 by the United Nations Office on Drugs and Crime (UNODC) of the Global Program for Combating Wildlife and Forest Crime (GPWLFC). NIRS technology was also introduced as one of five tools available in the paper published by the Global Timber Tracking Network (GTTN) in 2020 (Schmitz et al. 2020), which provides an overview of current analysis practices for wood identification.

Studies on the identification of the species *Aniba rosiodora* using NIRS technology have not been explored yet, neither studies on the characterization of the essential oil. However, NIRS technology can offer new lines of research, such as: identification of rosewood oil extracted from different parts of the plant and even verification of adulteration of essential oils.

3.8 TECHNOLOGIES OF PROPAGATION AND PRESERVATION OF POPULATIONS OF *Aniba rosiodora*

Rosewood is very important, from an economic point of view, for Amazon region, especially because of essential oil extraction. Even so, many trees are felled every year, causing a reduction of species *Aniba rosiodora* in its area of occurrence is Brazilian Amazon.

Disorderly exploration caused the social problem due to the reduction of rosewood extraction activity. It is estimated that around 6,000 people were directly involved in extractive practice, but many had to abandon the practice. In addition to the social problem, other problems are also a consequence of overexploitation. Despite several warnings, rosewood natural populations were practically decimated in Amazon Forest, with only a small number of remaining individuals concentrated in forest reserves. Among them, Adolpho Ducke Forest Reserve – Inpa/AM, Experimental Station of Cura-Uma/PA and glebe Camaçari/Silves-AM, which are the most significant (Contim and Contim 2018).

Despite the existence of remaining populations, this small number of individuals limits sources of genetic variability of the species to be used in selection and genetic improvement programs. Available information on genetic structure of the species and its diversity is rare, which is a negative factor (Contim and Contim 2018). Studies on genetic variability of the species are necessary and some have already started studies on the subject: Santos (2004) and Angrizani et al. (2013).

These authors found significant levels of genetic variability among individuals that are part of Adolpho Reserve population and other locations. Additionally, their studies showed that the information collected with researches and field technicians from forest reserves previously mentioned (Adolpho Ducke Forest Reserve – Inpa/AM, Experimental Station of Cura-Uma/PA and glebe Camaçari/Silves- AM) indicated the existence of three groups of individuals (ecotypes) of *Aniba rosiodora*, which would be phenotypically distinct due to physiological changes, morphology of some organs and terms of oil content. Possible groups of individuals are identified as “mulatinho”, “preciosa”, and “itaúba” (Bastos 1943). This information describes the variability of the species and directly affects the characteristics of the plant and, consequently, of the essential oil (final product).

Aniba rosiodora species also presents a natural limitation in reproduction, which aggravates the situation regarding populations. Due to this limitation, some practices for the recovery of the species are impaired, such as: planting implantation, replacement of natural reserves and rosewood exploration in rational extractive models (Contim and Contim 2018). Rosewood is a species that has low seed production, making its natural regeneration and establishment of *ex situ* populations even more difficult, as one of the viable alternatives for reducing pressure on natural populations that still exist (Handa et al. 2005).

Due to these problems, studies for species' conservation have been carried out since the 1990s. In 1999, Quisen and Handa (1999) studied the genetic variability existing within natural rosewood populations through the use of isoenzymes and they established a micro propagation protocol of the species to generates subsidies in the elaboration of conservation strategies.

Handa et al. (2005) also studied the *in vitro* establishment of embryos and rosewood seedlings buds without any contamination for species' propagation. As a result, sprouts showed 48% survival, which represents a good percentage. Therefore, the study showed that *in vitro* rosewood propagation becomes an alternative for conservation and use of species' economic potential, trough selected genotypes multiplication, providing the producer with seedlings for *ex situ* planting and promoting the enrichment of native forests.

In addition to *in vitro* cultures, rosewood propagation by cuttings and mini cuttings also presents promising studies. Preliminary tests indicated that juvenile rosewood cuttings showed high percentages of rooting compared to adult cuttings (Menezes 2006), which is an advantage, as cuttings are younger and can be obtained in less time. The vegetative rosewood propagation by cutting techniques and mini cuttings originated from juvenile material is possible and propagation via mini cuttings allows a higher percentage of rooting, survival and reduction of seedling time formation compared to conventional cutting method (Menezes et al. 2018).

Regarding the use of vegetative propagation by cuttings, this is also a viable alternative from a genetic point of view because it represents a guarantee that future trees will retain the same characteristics of source material (Handa et al. 2005). This is important because source material's characteristics are also related to essential oil production. If the source material produces a quality oil (e.g., with more linalool content), future trees are more likely to produce a quality oil.

Another perspective was also explored with studies by Freitas (2005). He found a good morphogenic response *in vitro* from shoot apexes. Shoot tip culture *in vitro* is a technique used to eliminate viruses and other pathogens in tree species. This methodology consists of shoot apical meristem, unexpanded leaves at various development stages, and a number of leaf primordia about 1 cm in length. In shoot-tip culture the explants are inoculated in cytokinin-supplemented media. Then manipulation of the shootlets is done in the rooting medium to develop plantlets. *In vitro* shoot-tip culture is widely used for clonal progenies that are phenotypically uniform and develop without chromosomal changes or meiotic irregularities (Bahtia et al. 2015).

In vitro shoot-tip culture resulted in the formation of a large number of shoots, a high *in vitro* rooting index and a formation of somatic embryos from friable calluses. Therefore, the technique proved to be an alternative for propagation and production of seedlings on a large scale, more viable in short-term. Freitas (2005, 2011) and Veras (2007) showed that rosewood can be satisfactorily propagated *in vitro* from shoot-tip culture, which when it's rooted can be transplanted into the soil, allowing large-scale production of seedlings in appropriate structure. Figure 23 illustrates that:

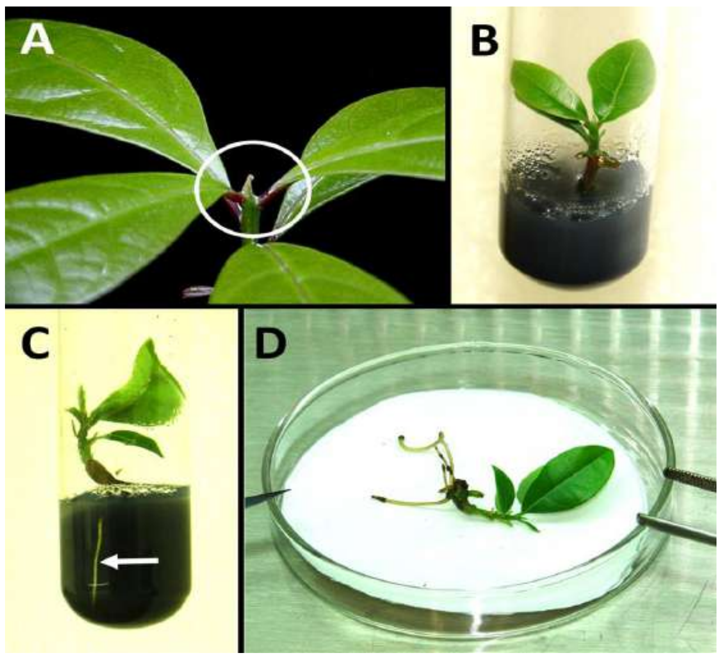


Figure 23: Rosewood seedling production *in vitro*. (A) Rosewood seedling obtained from seed germination. Steam apex is highlighted; (B) Aerial part development from shoot-tip culture; (C) Induction of root formation, indicated by the arrow and (D) Complete seedling obtained by shoot-tip culture, ready to be transferred to the soil (Contim and Contim 2018).

The technology of *in vitro* rosewood propagation generated from the development of the project "Integrated effort for conservation, genetic improvement and biotechnology of rosewood and related species: subsidies for sustainable development and rational exploitation of the species", coordinated by Luis Antônio Serrão Contim, enabled plant implantation of rosewood seedlings on a large scale. The first works carried out by the project covered communities in Extractive Forest Reserve of Silves (AVIVE, "Associação Vida Verde da Amazônia", Silves-AM – Brazil), in Flona Tapajós (Presidente Figueired/AM), in forest management area of Madeireira Mil in Itaquatiara/AM and in Apuí/AM, where there are still identified natural populations.

In addition to the reserves mentioned before, *Aniba rosiodora* is also found in Rosewood National Forest. In 2018, the president of Chico Mendes Intitute for Biodiversity Conservation approved a management plan for Rosewood Flona in the state of Amazonas. The forest covers an area of 988,186 hectares and is located in Maués and Nova Olinda do Norte (**Instituto Chico Mendes de Conservação da Biodiversidade - Flona de Pau-Rosa**. Icmbio.gov.br. <https://www.icmbio.gov.br/portal/flona-de-pau-rosa>). Figure 24 below shows some photographs of the forest:

Figure 24: Rosewood Flona (AM) (1) Seedling being cultivated, (2, 3) photographs of rosewood tree trunk taken for botanical record. The cut on the trunk is done to smell the aroma of the wood and check if the tree is really of *Aniba rosiodora* species and (3) rosewood trees planting (Caroline Schmaedeck Lara).

It is true to say that the remaining populations of rosewood have significant genetic variability and need to be preserved. Technologies for species' propagation are being developed and with appropriate investment, whether public and/or private, it may be possible to make rosewood planting systems viable on a large scale and the enrichment of natural populations. In addition, planting, managing natural populations and selling raw materials are also an important source of resources for Amazon region, including the activities of riverside communities and small producers (Contim and Contim 2018).

4 CONCLUSION AND RECOMMENDATIONS

The presented results showed that the species *Aniba rosiodora* stands out in extractive and economic scenario of Amazon region. Overvaluation of rosewood essential oil and interest in industry are proof of this. However, due to predatory exploitation, nowadays *Aniba rosiodora* is listed as endangered and therefore requires stricter regulation for trade. In this scenario, studies on species conservation, alternative extraction methods, propagation technologies and species control were discussed.

Traditional extraction method involves felling trees so that oil can be extracted from tree trunk. However, it is estimated that millions of trees have already been felled in this way and, thus, it is not a sustainable extraction method. Therefore, the importance of developing alternative techniques was highlighted. Linalool presence in oils extracted from leaves and branches allowed a new perspective regarding rosewood essential oil production. Oil extraction from leaves and branches comes up as an alternative to avoid cutting down trees (May AND Barata 2004) and, consequently, to preserve remaining natural populations. I suggest that further studies on commercial viability of rosewood oil from leaves and branches be developed.

Regarding conservation and control studies of the species, it was observed that establishment of rosewood plantations is presented as an alternative to replace oil extraction in natural population (Lara 2012). In addition, the technology of *in vitro* rosewood propagation proved to be viable for plants implantation for rosewood seedlings production on a large scale (Contim and Contim 2018). These studies showed to be promising for the protection of *Aniba rosiodora* remaining natural populations. Thus, it is necessary to continue studying the feasibility of applying these alternatives in field.

A high point of studies about *Aniba rosiodora* is related to NIR spectroscopy application. This technique has been shown to be useful for predicting rosewood essential oil yield (Amusant et al. 2016), making it feasible to predict oil yield without having to fell trees and even before extraction process. This would be interesting for industry and commerce, as it would be possible to pre-select potential individuals to produce oil with a greater amount of linalool.

NIRS technology (association of near-infrared spectroscopy and multivariate analysis) can also be applied to identify rosewood oil samples and supposed alterations, similar to what was done in the work by Souza et al. (2011). However, Souza et al. (2011) used direct infusion of samples via electrospray ionization and mass spectrometry (ESI-MS). The use of a portable NIR spectrometer together with principal component analysis (PCA) and/or other chemometric tools is a viable alternative for identifying adulteration in rosewood oil. This kind of analysis is extremely relevant so that buyers are not misled and it can also be applied in filed, once it is available a portable NIR equipment.

In addition, NIRS technology is used for identification/classification of forest species (Pastore et al. 2011, Braga et al. 2011, Bergo et al. 2016, Soares et al. 2017) and can be adapted to rosewood by building up a chemometric model. In this way, it is possible to identify species' wood in relation to other forest species as well as its origin (Silva et al. 2018).

It is worth emphasizing that the construction of an identification model involves obtaining a large amount of spectra of the studied species, as well as a proper application of chemometric tools. Thus, I suggest that to build a robust model for *Aniba rosiodora* species, spectra of samples should be collected in all countries where the species is found (French Guiana, Suriname, Guyana, Venezuela, Peru, Colombia and Ecuador). Besides offering a greater variability to sample set, this also allows the construction of a species database. These spectra can also be used to build a species discrimination model between different countries of occurrence, if it is interesting to identify the origin of wood (Silva et al. 2018).

Finally, with the information contained in this work, it was possible to obtain a historical and general overview of the technical-scientific production on rosewood oil in Brazil, which allows defining new steps to achieve the following project objective: build an exploratory model of identification of oil extracted from forest species *Aniba rosiodora*.

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