Article

Chemical Composition, Enantiomeric Distribution, and Antifungal Activity of the Oleoresin Essential Oil of *Protium amazonicum* from Ecuador

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Abstract: Background: *Protium* species (Burseraceae) have been used in the treatment of various diseases and conditions such as ulcers and wounds. Methods: The essential oil from the oleoresin of *Protium amazonicum* was obtained by hydrodistillation and analyzed by GC-MS, GC-FID, and chiral GC-MS. *P. amazonicum* oleoresin oil was screened for antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Cryptococcus neoformans*. Results: A total of 54 components representing 99.6% of the composition were identified in the oil. The essential oil was dominated by δ-3-carene (47.9%) with lesser quantities of other monoterpenoids α-pinene (4.0%), *p*-cymene (4.1%), limonene (5.1%), α-terpineol (5.5%) and *p*-cymen-8-ol (4.8%). Chiral GC-MS revealed most of the monoterpenoids to have a majority of *levo* enantiomers present with the exceptions of limonene and α-terpineol, which showed a *dextro* majority. *P. amazonicum* oleoresin oil showed promising activity against *Cryptococcus neoformans*, with MIC = 156 µg/mL. Conclusions: This account is the first reporting of both the chemical composition and enantiomeric distribution of the oleoresin essential oil of *P. amazonicum* from Ecuador. The oil was dominated by (−)-δ-3-carene, and this compound, along with other monoterpenoids, likely accounts for the observed antifungal activity of the oil.

Keywords: essential oil composition; *Protium amazonicum*; Burseraceae; copal; breu; δ-3-carene; chiral gas chromatography; antifungal activity

1. Introduction

*Protium amazonicum* (Cuatrec.) Daly belongs to the Burseraceae, which is comprised of 640 species representing 18 genera throughout the world, mainly distributed in the Neotropics and North Africa [1]. The main characteristic of the Burseraceae is the exuding aromatic resin [2,3], which is known as “copal” in Spanish [4] and “breu” in Portuguese [5]. *Protium* spp. have been used in the treatment of various diseases and conditions such as ulcers and wounds, to treat headaches, toothaches, and rheumatism [2], because of their anti-inflammatory [6,7], antinociceptive [8,9], antineoplastic [10], and gastroprotective [11,12] properties. The Yanomami people of Brazil use the resin of *P. fimbriatum* to treat respiratory infections [13]. *Protium* oleoresins have been characterized in terms of color, age, odor, as well as volatile and non-volatile chemical characteristics (Table 1) [5,14]. Because of the importance of *Protium* oleoresins in traditional medicine and because no previous work had been carried out on *P. amazonicum*, we wished to chemically characterize the oleoresin essential oil of *P. amazonicum*; this information should add to our understanding of *Protium* oleoresin chemistry.
Table 1. A brief review of *Protium* oleoresin traditional medicinal uses, biological properties, and essential oil compositions. *a*

| Species                      | Traditional Medicinal Uses and/or Biological Activities                                                                 | Major Components                                                                                     | Ref. |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------|
| *P. altsonii* (sucuruba)     |                                                                                                                          | *p*-cymene (16.3%), γ-cadinene (9.5%), γ-gurjunene (5.2%)                                           | [15] |
| *P. bahianum*                | Treatment of wounds, ulcers, inflammation, and as an insect repellent                                               | Fresh resin: *p*-cymene (18.3%), α-phellandrene (14.0%), tricyclene (11.4%), β-phellandrene (9.1%), β-pinene (6.6%) | [16] |
| *P. bahianum*                | Acaricidal activity (*Tetranychus urticae*)                                                                          | Aged resin: (E)-β-santalol acetate (83.1%)                                                          | [16] |
| *P. decandrum* (black breu)  | Antimicrobial (*Candida albicans*, MIC = 1.25 μg/mL; *Staphylococcus aureus*, MIC = 2.5 μg/mL)                         | α-pinene (10.5%), α-phellandrene (16.7%), *p*-cymene (6.0%), limonene (16.9%), terpinolene (28.5%) | [18] |
| *P. decandrum* (white breu)  | Antinociceptive (mouse model)                                                                                           | 1,8-cineole (58.7%), α-terpinene (13.7%), α-phellandrene (10.4%), γ-terpinol (7.7%)                   | [9]  |
| *P. heptaphyllum*            | Anti-inflammatory (rat model)                                                                                           | limonene (50.0%), (E)-β-ocimine (11.8%), 1,8-cineole (10.9%), α-phellandrene (10.8%)                | [7]  |
| *P. heptaphyllum*            | Anti-genotoxic activity                                                                                                 | terpinolene (32.7–37.8%), *p*-cymene (7.9–38.1%), limonene (0–2%), δ-3-carene (0–15.0%), α-thujene (0–1.1%), *p*-cymen-8-ol (2.5–10.1%) | [19] |
| *P. heptaphyllum*            |                                                                                                                          | Fresh resin: terpinolene (28.2–69.7%), *p*-cymene (4.3–23.3%), α-pinene (3.6–14.6%), α-terpinene (3.1–10.4%), limonene (6.4–10.1%), *p*-cymen-8-ol (2.7–9.8%) | [20] |
| *P. heptaphyllum*            |                                                                                                                          | Aged resin: *p*-cymene (18.7–43.0%), terpinolene (8.8–21.6%), α-pinene (3.5–17.8%), α-limonene (5.8–1.6%), *p*-cymen-8-ol (8.2–31.8%) | [20] |
| *P. heptaphyllum*            |                                                                                                                          | Fresh resin: myrcene (35.0%), α-pinene (27.0%), sabinen (11.0%), β-caryophyllene (7.2%)                | [10] |
| *P. heptaphyllum*            | Cytotoxic on SP2/0 (murine plasmocytoma) and J774 (murine monocytic macrophage) cell lines                           | Freshly tapped resin: terpinolene (28.0%), *p*-cymene (16.0%), α-pinene (8.7%), α-terpinene (6.6%), limonene (5.5%), *p*-cymen-8-ol (5.6%) | [10] |
| *P. heptaphyllum*            | Antibacterial (*Streptococcus mutans*, MIC 0.13 μg/mL)                                                                   | tricyclene (11.1%), *p*-cymene (26.7%), terpinolene (35.8%), *p*-cymen-8-ol (10.1%)                  | [21] |
| *P. heptaphyllum*            | Vasorelaxant (rat upper mesenteric artery ring, *IC*<sub>50</sub> 316 μg/mL)                                             | δ-3-carene (5.1%), *p*-cymene (17.0%), limonene (34.5%), 1,8-cineole (20.6%), α-terpinol (9.8%)       | [22] |
| *P. heptaphyllum*            |                                                                                                                          | α-phellandrene (7.0%), *p*-cymene (26.9%), limonene (28.9%), α-terpinol (18.4%)                       | [22] |
| *P. heptaphyllum*            |                                                                                                                          | Fresh resin: α-terpinene (18.0%), *p*-cymene (36.0%), γ-terpinene (12.0%)                            | [25] |
| *P. heptaphyllum*            | Aged resin: *p*-cymene (11.0%), terpinolene (15.0%), *p*-cymene (5.3%), *p*-cymen-8-ol (11.0%), dillapiole (16.0%)    |                                                                                                      | [23] |
| *P. heptaphyllum*            |                                                                                                                          | Fresh resin: α-pinene (10.5%), α-phellandrene (16.7%), *p*-cymene (6.0%), limonene (16.9%), terpinolene (28.5%) | [24] |
| *P. heptaphyllum* (black breu) | Treatment of headaches (inhalation); treat pain and inflammation (plasters)                                         |                                                                                                      | [25] |
| *P. heptaphyllum* (black breu) |                                                                                                                          | δ-3-carene + iso-sylvestrene (79.5%)                                                               | [15] |
| *P. heptaphyllum* (black breu) |                                                                                                                          | δ-3-carene + iso-sylvestrene (56.4%), *p*-cymene (14.0%), limonene + β-phellandrene (6.8%)           | [15] |
| *P. heptaphyllum* (black breu) |                                                                                                                          | *p*-cymene (33.0%), δ-3-carene + iso-sylvestrene (14.7%)                                         | [15] |
Table 1. Cont.

| Species                  | Traditional Medicinal Uses and/or Biological Activities                                             | Major Components                                                                 | Ref.   |
|--------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------|
| P. heptaphyllum          | Traditional remedy for inflammations, as an inhalant to clear respiratory and bronchial passages, wound healing. Antibacterial, disk diffusion assay (Bacillus subtilis, Staphylococcus aureus) | Terpinolene (42.3%), p-cymen-8-ol (13.6%), limonene (11.9%)                        | [26]   |
| P. heptaphyllum subsp.  |                                                                                                 | P-cymene (39.9%), n-tetradecane (13.4%), dihydro-4-carene (11.7%), α-phellandrene (7.4%) | [26]   |
| P. heptaphyllum subsp.   |                                                                                                 | P-cymene (6.4%), limonene + β-phellandrene (5.7%)                                 | [15]   |
| P. heptaphyllum subsp.   |                                                                                                 | P-cymene (20–40%), limonene (5.8–8.0%), α-terpinolene (5.8–31%), p-cymen-8-ol (10–26%) | [27]   |
| P. icicariba             |                                                                                                 | α-pinene (5.6–7.7%), p-cymene (5.6–7.7%), limonene (5.8–8.0%), α-terpinolene (5.8–31%), p-cymen-8-ol (10–26%) | [27]   |
| P. neglectum             |                                                                                                 | Traditional remedy for inflammations, as an inhalant to clear respiratory and bronchial passages, wound healing. Antibacterial, disk diffusion assay (Bacillus subtilis, Staphylococcus aureus) | [28]   |
| P. occultum (white breu) | Burning and inhaling smoke to treat headache                                                   | Fresh resin: p-cymene (5.2%), durenol (15.6%), α-terpinol (6.9%), pipertinone (25.4%), thymol (17.5%), methyl eugenol (9.2%) | [15]   |
| P. cf. opicum (surucuba) | Traditional remedy for inflammations, as an inhalant to clear respiratory and bronchial passages, wound healing. Antibacterial, disk diffusion assay (Bacillus subtilis, Staphylococcus aureus) | α-pinene (6.6%), α-neo-cloveene (5.3%), α-neo-callitropsene (7.3%), γ-cadinene (14.4%) | [15]   |
| P. strumosum (white breu) | Burning and inhaling smoke to treat headache                                                   | α-pinene (57.7%), β-pinene (9.3%), p-cymene (9.2%), limonene + β-phellandrene (10.8%) | [15]   |

* Rüdiger and co-authors have reviewed the chemistry and pharmacology of Protium in 2007 [2]. This table includes analyses reported since 2007.

2. Materials and Methods

2.1. Essential Oil

The oleoresin (relatively fresh, yellow, with a terpenic odor) of P. amazonicum was collected from Quito, Ecuador (0°14′0″ S, 78°31′0″ W, 3000 m above sea level). The tree was identified by Rafael Parducci, and a voucher specimen has been deposited in Saintoil S.A. The essential oil was obtained by hydrodistillation using a Clevenger apparatus as previously described [29] to give the essential oil.

2.2. Gas Chromatography-Mass Spectrometry (GC-MS)

The oleoresin essential oil of P. amazonicum was analyzed by GC-MS using a Shimadzu GC-MS-QP2010 Ultra (Shimadzu Corp., Columbia, MD, USA) operated in the electron impact (EI) mode (electron energy = 70 eV), with a scan range of 40–400 atomic mass units (amu), a scan rate of 3.0 scans/s, and the GC-MS Solution Software (Shimadzu GC-MS-QP2010 Ultra, Columbia, MD, USA). The GC column was ZB-5MS fused silica capillary column (Phenomenex Inc., Torrance, CA, USA) (30 mL × 0.25 mm ID) with a (5% phenyl)-polydimethylsiloxane stationary phase with a film thickness of 0.25 μm. The carrier gas was helium with a column head pressure of 551.6 kPa and flow rate of 1.37 mL/min. The injector temperature was 250 °C, and the ion source temperature was 200 °C. The GC oven temperature program was programmed for 50 °C initial temperature, the temperature increased at a rate of 2 °C/min to 260 °C. A 5% w/v solution of the sample in CH₂Cl₂ was prepared and 0.1 μL was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of n-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [30], and stored in the MS library.

2.3. Gas Chromatography—Flame Ionization Detection

The gas chromatograph was a Shimadzu GC 2010 (Shimadzu Corp., Columbia, MD, USA) equipped with a flame ionization detector, a split/splitless injector, and autosampler AOC-20i.
The clear pale yellow oleoresin essential oil from *P. amazonicum* was obtained in 0.3% yield and analyzed by GC-MS and GC-FID. From a total of 56 peaks, 99.6% of the compounds were identified in the oil (Table 2). The major components of the resin oil were identified as δ-3-carene (47.9%), α-pinene (4.0%), *p*-cymene (4.1%), limonene (5.1%), α-terpineol (5.5%) and *p*-cymen-8-ol (4.8%) (see Figure 1). δ-3-Carene has been reported as a major component in several Protium spp. oleoresin essential oils, including *P. decandrum* and *P. heptaphyllum* [15]; however, in most oleoresin essential oils from Protium, δ-3-carene is a minor component or unobserved (see Table 1). *Protium* oleoresin
oils show wide variation in chemical composition, depending on species as well as age and color of the resin (Table 1). The age of an oleoresin has a distinct effect on the chemical composition. Some monoterpenes have been found to undergo oxidation upon exposure to atmospheric oxygen [33–35], including oleoresin monoterpenoids [20]. In addition, fresh oleoresin from the same species shows wide variation in chemical composition. Thus, for example, the essential oil from fresh oleoresin of *P. heptaphyllum* collected from the Restinga of Carapebus, Rio de Janeiro state, Brazil, had myrcene (35.0%) and α-pinene (27.0%) as the major components [10]; the fresh resin oil from Reserva da Campina, Amazonas, Brazil, was rich in *p*-cymene (36.0%), α-terpinene (18.0%), and γ-terpinene (12.0%) [23]; and the fresh resin oil from Crato, Ceara, Brazil was dominated by terpinolene (28.5%), α-phellandrene (16.7%), and limonene (16.9%) [24]. The oleoresin in this present work is a relatively fresh resin, reflected in the high concentration of δ-3-carene.

### Table 2. Chemical composition of the oleoresin essential oil of *Protium amazonicum* from Ecuador.

| RI<sup>c</sup>calc | RI<sup>lit</sup> | Compound                              | %     |
|-------------------|----------------|---------------------------------------|-------|
| 779               | 780            | Toluene                               | 0.2   |
| 925               | 930            | α-Thujene                             | 0.7   |
| 932               | 939            | α-Pinene                              | 4.0   |
| 947               | 952            | α-Fenchene                            | 0.2   |
| 949               | 954            | Camphene                              | 0.1   |
| 970               | 972            | 3,7,7-Trimethyl-1,3,5-cycloheptatriene | 1.4   |
| 972               | 975            | Sabinene                              | 1.0   |
| 977               | 979            | β-Pinene                              | 1.0   |
| 1000              | 1002           | δ-2-Carene                            | 0.1   |
| 1007              | 1002           | α-Phellandrene                         | 0.5   |
| 1010              | 1011           | δ-3-Carene                            | 47.9  |
| 1017              | 1017           | α-Terpinene                           | 0.4   |
| 1019              | 1026           | o-Cymene                              | 0.3   |
| 1024              | 1024           | *p*-Cymene                            | 4.1   |
| 1029              | 1029           | Limonene                              | 5.1   |
| 1030              | 1029           | β-Phellandrene                         | 0.4   |
| 1032              | 1031           | 1,8-Cineole                           | 0.7   |
| 1057              | 1059           | γ-Terpinene                           | 0.5   |
| 1072              | 1072           | Pinol                                 | 0.2   |
| 1080              | 1085           | *m*-Cymene                            | 1.8   |
| 1085              | 1088           | Terpinolene                           | 0.7   |
| 1090              | 1091           | *p*-Cymene                            | 3.2   |
| 1095              | 1099           | α-Pinene oxide                        | 0.1   |
| 1141              | 1139           | *trans*-Pinocarveol                   | 0.1   |
| 1142              | —              | 2-Isobutyl-norbornane                 | 0.7   |
| 1147              | 1146           | Camphor                               | 0.3   |
| 1149              | 1147           | *trans*-Dihydro-α-terpineol           | 0.5   |
| 1153              | 1150           | Eucarvone                             | 0.3   |
| 1162              | 1170           | α-Phellandren-8-ol                    | 1.9   |
| 1170              | 1160           | *iso*-Borneol                         | 0.3   |
| 1171              | —              | β-Phellandren-8-ol                    | 0.9   |
| 1174              | 1169           | Borneol                               | 0.6   |
| 1180              | 1179           | *m*-Cymen-8-ol                        | 4.8   |
| 1183              | —              | *p*-Isobutyltoluene                   | 0.3   |
| 1184              | 1182           | *p*-Methylacetophenone                | 0.1   |
| 1186              | 1182           | *p*-Cymen-8-ol                        | 1.7   |
| 1188              | —              | (*Z*)-β-Ocemienol                     | 0.2   |
| 1195              | 1188           | α-Terpinol                            | 5.5   |
| 1207              | 1205           | Verbenone                             | 0.2   |
| 1210              | 1217           | 4-Methyleneisophorone                 | 3.0   |
| 1220              | —              | 2-Carone                              | 0.9   |
| 1240              | 1238           | (E)-Ocimenone                         | 0.2   |
| 1242              | 1241           | Cuminal                               | 0.1   |
The (+)-enantiomer of limonene is the more common, especially in *Citrus P. amazonicum* present in *Pinus sylvestris* detected in the other hand, were dominant. The hexane root extract of *Angelica archangelica* and sabinene predominated over the (+)-isomers. The (+)-enantiomers of limonene and α-pinene, β-pinene, thujene, and α-cymene showed predominantly (+)-3-carene, but the (−)-enantioomer of δ-3-carene was not determined [45]. In *Boswellia carterii* (Burseraceae) from Ethiopia was composed of (+)-limonene, (+)-α-pinene, and (−)-limonene, but the enantiomeric distribution of δ-3-carene was not determined [46]. In *Laurus nobilis* (Lauraceae) essential oil (see Table 3 and Figure 2). The levorotatory (−)-enantiomer was detected [36]. Only the (+)-enantiomer of δ-3-carene was detected in *Pinus sylvestris* (Pinaceae) essential oils, while (−)-limonene predominated [37]. The (+)-enantioomer of limonene is the more common, especially in *Citrus* (Rutaceae) essential oils [38–42].

### Table 2. Cont.

| Retention Index (RI) | Compound | % |
|----------------------|----------|---|
| 1243 1243            | Carvone  | 0.2 |
| 1246 1248            | Car-3-en-2-one | 0.4 |
| 1248 1247            | Carvotanacetone | 0.2 |
| 1253 1252            | Piperitone | 0.1 |
| 1264 1268            | 3,5-Dimethoxytoluene | 0.2 |
| 1277 1275            | Phellandranal | 0.3 |
| 1290 1290            | Thymol | 0.2 |
| 1296 1299            | Carvacrol | 0.3 |
| 1419 1419            | β-Caryophyllene | 0.1 |
| 1433 1434            | α-trans-Bergamotene | 0.9 |
| 1581 1583            | Caryophyllene oxide | 0.2 |
|                      | Total identified | 99.6% |

RI\(^{calc}\) = Retention indices calculated in reference to a homologous series of n-alanes on a ZB-5MS column. RI\(^{lit}\) = Retention indices from the literature [30].

![Gas chromatogram of the oleoresin essential oil of Protium amazonicum from Ecuador.](image)

**Figure 1.** Gas chromatogram of the oleoresin essential oil of *Protium amazonicum* from Ecuador. 1, α-thujene; 2, α-pinene; 3, 3,7,7-trimethyl-1,3,5-cycloheptatriene; 4, β-pinene; 5, δ-3-carene; 6, p-cymene; 7, limonene; 8, m-cymenene; 9, p-cymenene; 10, α-phellandren-8-ol; 11, m-cymen-8-ol; 12, p-cymen-8-ol; 13, α-terpineol; 14, 4-methyleneisophorone; 15, α-trans-bergamotene; 16, caryophyllene oxide.

#### 3.2. Enantiomeric Distribution

Chiral GC-MS analysis was performed to evaluate the enantiomeric distribution of the monoterpenes present in *P. amazonicum* essential oil (see Table 3 and Figure 2). The levorotatory (−)-enantioomer of δ-3-carene was found to be the exclusive stereoisomer while the (−)-enantioomers of α-pinene, β-pinene, and sabinene predominated over the (+)-isomers. The (+)-enantioomers of limonene and α-terpineol, on the other hand, were dominant. The hexane root extract of *Angelica archangelica* showed predominantly (+)-δ-3-carene, but the (−)-enantioomer was detected [36]. Only the (+)-enantioomer of δ-3-carene was detected in *Pinus sylvestris* (Pinaceae) essential oils, while (−)-limonene predominated [37]. The (+)-enantioomer of limonene is the more common, especially in *Citrus* (Rutaceae) essential oils [38–42].
Micromeria fruticosa (Lamiaceae) essential oil showed exclusively (+)-α-terpineol while (−)-α-terpineol was found in Laurus nobilis (Lauraceae) essential oil [43]. Analysis of the essential oil from the unripe fruits of Pistacia vera showed a predominance of (+)-α-pinene, (+)-limonene, (+)-β-pinene, and exclusively (−)-α-terpineol [44]. Although δ-3-carene was relatively abundant in this oil (2.7%), the enantiomeric distribution was unfortunately not reported. The oleoresin of Boswellia carterii (Burseraceae) from Ethiopia was composed of (+)-α-thujene, (−)-α-pinene, and (−)-limonene, but the enantiomeric distribution of δ-3-carene was not determined [45]. In contrast, B. carterii resin oil from Somalia showed (−)-α-thujene, (−)-α-pinene, and (−)-limonene predominating, while B. sacra resin oil from Oman had (+)-α-thujene, (+)-α-pinene, (+)-β-pinene, and (−)-limonene predominating [46].

Table 3. Enantiomeric excess (ee) and distribution (ed) of monoterpenoids in the resin oil of Protium amazonicum.

| Compounds       | Relative % | ee (%) | ed [(+) to (−)] (%) |
|-----------------|------------|--------|---------------------|
| α-Thujene       | 0.7        | 45.6   | 27.2 to 72.8        |
| α-Pinene        | 4.0        | 41.8   | 29.1 to 70.9        |
| β-Pinene        | 1.0        | 45.6   | 27.2 to 72.8        |
| δ-3-Carene      | 47.9       | 100    | 0 to 100            |
| Limonene        | 5.1        | 68.0   | 84.0 to 16.0        |
| α-Terpineol     | 5.5        | 79.6   | 89.8 to 10.2        |

Figure 2. Chiral gas chromatogram of the oleoresin essential oil of Protium amazonicum. 1, (+)-α-thujene; 2, (−)-α-thujene; 3, (+)-α-pinene; 4, (−)-α-pinene; 5, (+)-β-pinene; 6, (−)-β-pinene; 7, 3,7,7-trimethyl-1,3,5-cycloheptatriene; 8, 1,8-cineole; 9, (−)-δ-3-carene; 10, (−)-limonene; 11, (+)-limonene; 12, p-cymene; 13, α-terpinolene; 14, γ-terpinene; 15, m-cymenene; 16, p-cymene; 17, camphor; 18, α-phellandren-8-ol; 19, (−)-α-terpineol; 20, eucarvone; 21, (+)-α-terpineol; 22, m-cymen-8-ol; 23, p-cymen-8-ol; 24, α-trans-bergamotene.

3.3. Antifungal Activity

The oleoresin essential oil of P. amazonicum demonstrated antifungal activity against C. albicans, C. neoformans, and A. niger. C. neoformans was most potently inhibited with a promising MIC of 156 μg/mL. Inhibition of C. albicans (MIC = 313 μg/mL) was also rather promising whereas inhibition of A. niger was relatively weak (MIC = 1250 μg/mL). The major component in P. amazonicum oil,
δ-3-carene, has shown antifungal activity against several fungi, including C. albicans [47]. In addition, minor monoterpenoid components in the oil, α-pinene [48], limonene [49], and α-terpineol [50], have also shown antifungal activities.

The antifungal mechanisms of activity of monoterpenoids are poorly understood. It has been suggested that these hydrophobic compounds disrupt the cytoplasmic membranes or membrane proteins of fungal cells, leading to cytoplasmic leakage, cell lysis, and death [51]. Chirality of monoterpenoids, therefore, may not play a critical role in antimicrobial activity. Nevertheless, Kusumoto and co-workers have shown that (+)-α-pinene showed significantly better antifungal activity against Heterobasidion parviporum than (−)-α-pinene [52]. Likewise, Filipowicz et al. showed (−)-β-pinene to be slightly more active than (+)-β-pinene against Candida albicans [53], and Omran and co-workers found that (−)-limonene had better antifungal activity than (+)-limonene [54].

(+)δ-3-Carene has shown antifungal activity against several fungal strains [47], but there are apparently no reports on antifungal activity of (−)δ-3-carene, which is not commercially available. Overall, these findings indicate that P. amazonicum resin oil has promising potential for further antifungal consideration, in particular against C. neoformans and potentially other yeast-like fungi.

4. Conclusions

This is the first reported chemical analysis of the oleoresin essential oil of Protium amazonicum. The P. amazonicum resin oil collected in Ecuador was dominated by (−)-δ-3-carene and is therefore, an excellent source of this enantiomer. The abundance of this compound, along with other monoterpenoids, likely account for the observed antifungal activity of the oil. The activity against Cryptococcus neoformans and Candida albicans indicates promise against these opportunistic fungal pathogens. Additional research into this tree species and other Protium species, their chemistry and their biological activities, is needed.

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