Phytochemical Profile of *Wollemia nobilis* Half-Matured Female Cones and Their Potential Ethnopharmacological and Nutraceutical Activities

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Abstract: In this work, the half-matured female cones of the rare species, *Wollemia nobilis*, were studied for their phytochemical profile for the first time. Fourteen compounds were isolated and identified by means of column chromatography, Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS). In particular, these compounds were acetyl-isocupressic acid (1), methyl-(E)-communate (2), sandaracopimaric acid (3), wollemol (4), 7″-O-methyl-agathisflavone (5), 7,4‴-di-O-methyl-agathisflavone (6), shikimic acid (7), quinic acid (8), glucose (9), sucrose (10), raffinose (11), D-lactic acid (12), succinic acid (13) and alanine (14). The chemotaxonomic implications of their presence were discussed and a preliminary phytochemical comparison between these cones and the male ones was also performed. This evidenced several similarities but also some differences that were widely treated about. Moreover, a preliminary nutraceutical evaluation of these cones, based on phytochemistry, was carried out. Actually, this showed a good nutraceutical potentiality of the half-matured cones but also some potential critical state mainly due to the occurrence of acetyl-isocupressic acid (1), which is quite known to have some adverse pharmacological effects. For this reason, more in-depth nutraceutical studies would be necessary to exactly determine the ethnopharmacological and nutraceutical value of these cones.

Key words: *Wollemia nobilis*, half-matured female cones, phytochemical analysis, primary and secondary metabolites, chemotaxonomy, ethnopharmacology, nutraceutics.

1. Introduction

Araucariaceae is a family of coniferous trees belonging to the Pinales order and to the Gymnosperms group. This family comprises 32 species distributed in three genera *Agathis* Salisb., *Araucaria* Juss. and *Wollemia* W.G. Jones, K.D. Hill & J.M. Allen. The species belonging to this family are morphologically characterized by an ever-green bearing. They can reach up to 65 m height and present a generally dioecious bearing. Leaves are simple and with variable shapes whereas the cones are more or less erect and heavy. Lastly, the seeds are very big [1]. These species are typical of the Southern Hemisphere from South-Eastern Asia to Australia, New Zealand and South America and can be prevalently found in tropical and sub-tropical forests [1].

Within the family, the genus *Wollemia* represents a quite rare element with only a few exemplars living in the wild. It is constituted by the only species, *Wollemia nobilis* W.G. Jones, K.D. Hill & J.M. Allen, and it was thought to be extinct several hundreds of years ago. Indeed, a little population of this species (20 large trees up to 40 m high and 20 juvenile trees) was discovered in 1994 in Australia. The species is also important since its adult foliage is comparable with fossils found in New South Wales and derived from the Jurassic Era. Its pollen is identical to those dated back from ninety to two millions years ago [2]. For this reason, this species...
is considered to be a living fossil and the research on it is now started again. From the botanical standpoint, this monoecious species is characterized by a brown bark. The stem is multiple with a complex root system. The branching is vertical and lateral. The leaves are flattened and there are two different cones. The male ones (the pollen) are conic and large with a brown-reddish color. The female ones (the seeds) are lighter in color and narrower. These cones are disposed in lower positions than the male cones [3]. The half-matured female cones present, instead, a conical shape with a pronounced elongation of its top and a brownish coloration (Fig. 1).

Due to its rarity, this species has been subjected to several protection and conservation programs implying an on-going project of cultivation and commercialization of Wollemi pine trees worldwide. For this reason, nowadays, several exemplars are now hosted in botanical gardens and in private house gardens all around the world [4].

In literature, only a few works are present concerning previous phytochemical analysis of this species, in particular, about the essential oil [5, 6] and the polar fraction content of the leaves and of the male cones [2, 7, 8].

In continuation with other comprehensive phytochemical studies on this species [2, 8], the attention was focused, this time, on the half-matured female cones of which the content in primary and secondary metabolites was studied. About this specific organ, no previous study has ever been conducted. The aims of this work were also: to perform a first phytochemical comparison between these cones and the male ones; to start the evaluation, from the phytochemical standpoint, of their potential ethnopharmacological and nutraceutical benefits as it has been already done with the male cones [2] since all these cones are widely eaten by animals; to verify if also in these organs a differential accumulation of metabolites exists like in the leaves and in the male cones [2, 8].

2. Materials and Methods

2.1 Plant Materials

Twenty-five grams (g) of half-matured female cones of *W. nobilis* were collected in the Botanical Garden of Rome located in Largo Cristina di Svezia 4, Rome (Italy) (geographical coordinates: 41°53′32″ N, 12°27′57″ E). An exemplar of this species has been planted in this botanical garden and directly derives from Wollemi National Park (Australia) where it was purchased in 2006. The botanical recognition was performed by the botanists of the garden even by

Fig. 1 *Wollemia nobilis* (left) and particular of the half-matured female cones (right).
comparison with the data available in the literature [3].

2.2 Chemicals

During this study, 96% ethanol and distilled water were utilized for the extraction procedure. Indeed, n-butanol, distilled water, dichloromethane and methanol as pure solvents or in mixtures among them all at different concentrations were used for the column chromatography separations on silica gel (40-63 μm) as stationary phase. Deuterated solvents, such as CDCl₃, CD₃OD and D₂O were employed for the identification of metabolites by means of Nuclear Magnetic Resonance (NMR) spectroscopy. Lastly, methanol having High Pressure Liquid Chromatography (HPLC) grade was used for the identification of metabolites by means of Mass Spectrometry (MS).

All the solvents having Analytical Grade Reagents (RPE) grade, if not differently specified, together with the deuterated solvents and the methanol with HPLC purity grade were purchased from Sigma-Aldrich while silica gel was purchased from Fluka Analytical.

2.3 Instrumentation

NMR spectra were recorded on a Varian (now Agilent Technologies) Mercury 300 MHz instrument and/or on a Bruker Avance III 400 MHz instrument with chemical shifts expressed in ppm. The chemical shifts were expressed from tetramethylsilane (TMS) (s, 0 ppm) for spectra in CDCl₃, the internal solvent signal of CD₂HOD (p, 3.31 ppm) was the reference for spectra in CD₃OD while the HDO signal (s, 4.79 ppm) was set as reference for spectra in D₂O.

MS spectra were performed on a Q-TOF MICRO spectrometer (Micromass, now Waters, Manchester, UK) equipped with an Electro-Spray Ionization (ESI) source operating in the negative and/or positive ion mode. The flow rate of sample infusion was 20 μL/min with 50 acquisitions per spectrum. Data were analyzed by using the MassLynx software developed by Waters.

2.4 Extraction Procedure

The whole 25.0 g of the half-matured female cones were inserted in a flask and covered with an extraction solution (about 150 mL) constituted by 96% ethanol and distilled water in ratio 80:20 (v/v) until their complete immersion. The cones were macerated for 48 h so that metabolites could come into solution. The obtained brownish solution was filtrated and the solvents were eliminated at reduced pressure at a temperature of 60 °C. Throughout concentration, pH was checked on litmus paper and this was about 6.5 pH units. This passage is necessary in order to verify that pH is not too acid or basic (meaning between the range 5.5-8.5) because an extreme acidity or alkalinity might cause secondary reactions in the extract such as the hydrolysis of ester and glycosidic bonds. A second and third extraction was also accomplished using only ethanol 96% as extraction solvent. The procedure was repeated exactly as previously described and the three solutions were collected altogether. The obtained final water suspension was then frozen at a temperature of -20 °C and later lyophilized to preserve also temperature-sensitive compounds eventually present. The resulting dried crude extract weighed 2.5 g and was light brown colored.

2.5 Isolation and Identification of Metabolites

A portion of 2.0 g of the dried crude extract underwent a first chromatographic separation with a corresponding amount of silica gel of 80.0 g (ratio 1:50 w/w) and n-butanol saturated with distilled water (82:18, v/v) as eluting system. From this first chromatographic separation nine compounds were isolated and identified by comparison with data reported in literature and standard compounds present in the laboratory: glucose (9) [9], sucrose (10) [9] and raffinose (11) [9] as an only mixture in ratio 4:1:1 from the assembly of fractions 34-68 for the total weight of 238.7 mg; shikimic acid (7) [10], quinic acid (8) [11], D-lactic acid (12) [9], succinic acid (13)
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[12] and alanine (14) [12] as an only mixture in ratio not detectable from the assembly of fractions 76-88 for the total weight of 160.9 mg.

Since not all the present metabolites could be clearly isolated and identified from this first step, a second chromatographic separation was performed on an assembly of fractions deriving from the first separation, 1-18, for the total weight of 1.0 g. The amount of silica gel was 40.0 g (ratio 1:40 w/w) and the elution system was a mixture of dichloromethane and methanol at different concentrations. The initial one was 98:2 (v/v) but, during the chromatographic run, this was gradually modified in order to raise the polarity and let the elution of more polar compounds, passing to 95:5 (v/v), 9:1 (v/v), 8:2 (v/v), 7:3 (v/v) and, lastly, 6:4 (v/v). From this chromatographic step, six further metabolites were isolated and identified by comparison with data reported in literature and standard compounds present in the laboratory: acetyl-isocupressic acid (1) [2], methyl-(E)-commenate (2) [2], sandaracopimaric acid (3) [2] and wollemol (4) [2] as an only mixture in ratio 3:2:1.5:1 from the assembly of fractions 7-9 for the total weight of 201.0 mg; acetyl-isocupressic acid (1) [2] and sandaracopimaric acid (3) [2] in mixture in ratio 1:2 from the assembly of fractions 10-13 for the total weight of 101.6 mg; 7''-O-methyl-agathisflavone (5) [13] and 7,4''-di-O-methyl-agathisflavone (6) [2] in mixture in ratio 3:1 from the assembly of fractions 41-62 for the total weight of 18.1 mg.

2.6 Quantification of the Identified Metabolites

The total exact quantification of the identified metabolites is reported in Table 1.

2.7 NMR Data of the Newly Isolated Metabolites in the Genus

7''-O-methyl-agathisflavone (5): $^1$H-NMR (CD$_3$OD, 400 MHz) $\delta$: 7.86 (2H, d, $J = 8.2$ Hz, H-2' and H-6'), 7.49 (2H, d, $J = 8.9$ Hz, H-2'' and H-6''), 6.72 (1H, s, H-8), 6.57 (1H, s, H-3''), 6.35 (1H, s, H-6''), 3.83 (3H, s, MeO-7').

ESI-MS: m/z 553.19 [M+H]$^+$.  
Quinic acid (8): $^1$H-NMR (400 MHz, D$_2$O) $\delta$: 4.24-4.19 (1H, m, H-4), 3.99-3.96 (overlapped signal, H-5), 3.61-3.56 (1H, m, H-3), 2.07-1.97 (2H, m, Ha-2, Hb-2), 1.87-1.83 (2H, m, Ha-6, Hb-6).

Table 1  Total quantification of the identified compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-isocupressic acid (1)</td>
<td>115.7</td>
</tr>
<tr>
<td>Methyl-(E)-commenate (2)</td>
<td>53.6</td>
</tr>
<tr>
<td>Sandaracopimaric acid (3)</td>
<td>107.9</td>
</tr>
<tr>
<td>Wollemol (4)</td>
<td>26.8</td>
</tr>
<tr>
<td>7''-O-methyl-agathisflavone (5)</td>
<td>13.57</td>
</tr>
<tr>
<td>7,4''-di-O-methyl-agathisflavone (6)</td>
<td>4.53</td>
</tr>
<tr>
<td>Shikimic acid (7)</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Quinic acid (8)</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Glucose (9)</td>
<td>159.1</td>
</tr>
<tr>
<td>Sucrose (10)</td>
<td>39.8</td>
</tr>
<tr>
<td>Raffinose (11)</td>
<td>39.8</td>
</tr>
<tr>
<td>D-lactic acid (12)</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Succinic acid (13)</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Alanine (14)</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>
ESI-MS: $m/z$ 191.32 [M-H]⁻.

Raffinose (11): $^1$H-NMR (400 MHz, D₂O) δ: 5.40 (1H, d, $J = 3.8$ Hz, H-1'), 4.98 (1H, d, $J = 3.7$ Hz, H-1), 4.20 (1H, d, $J = 8.7$ Hz, H-2''), 4.02 (3H, m, Ha-5', Ha-6'), 3.91 (1H, m, H-5), 3.88 (2H, m, H-4'' and H-2), 3.83 (1H, m, H-3'), 3.78 (3H, m, H-6 and H-3''), 3.71 (1H, m, Hb-6'), 3.68 (2H, s, H-6''), 3.57 (1H, m, H-2).

ESI-MS: $m/z$ = 527.24 [M+Na]⁺; $m/z$ = 503.29 [M-H]⁻.

D-lactic acid (12): $^1$H-NMR (400 MHz, D₂O) δ: 4.09 (1H, m, H-α), 1.34 (3H, d, $J = 6.9$ Hz, CH₃).

ESI-MS: $m/z$ = 89.12 [M-H]⁻.

Succinic acid (13): $^1$H-NMR (400 MHz, D₂O) δ: 2.44 (4H, s, α-CH₂ and β-CH₂).

ESI-MS: $m/z$ = 117.421 [M-H]⁻.

Alanine (14): $^1$H-NMR (400 MHz, D₂O) δ: 3.79 (1H, m, H-α), 1.47 (3H, d, $J = 7.3$ Hz, CH₃).

ESI-MS: $m/z$ = 88.13 [M-H]⁻.

3. Results and Discussion

The phytochemical analysis of the polar fraction of the half-matured female cones of *W. nobilis* led to isolation and identification of 14 compounds: acetyl-isocupressic acid (1), methyl-(E)-communate (2), sandaracopimaric acid (3), wollemol (4), 7''-O-methyl-agathisflavone (5), 7,4'''-di-O-methyl-agathisflavone (6), shikimic acid (7), quinic acid (8), glucose (9), sucrose (10), raffinose (11), D-lactic acid (12), succinic acid (13) and alanine (14) (Fig. 2).

These compounds belong to seven different classes of organic natural compounds such as diterpenes (1-4), biflavonoids (5-6), cyclohexencarboxylic acids (7), cyclohexancarboxylic acid (8), saccharides (9-11), natural organic acids (12-13) and amino acids (14).

All of them represent new metabolites for these
specific organs of the species whereas 7''-O-methyl-agathisflavone (5), quinic acid (8), raffinose (11), D-lactic acid (12), succinic acid (13) and alanine (14) have been identified for the first time in the genus during this study. On the other hand, acetyl-isocupressic acid (1), methyl-(E)-communate (2), sandaracopimarc acid (3), wollemol (4), 7-4'''-di-O-methyl-agathisflavone (6), shikimic acid (7), glucose (9) and sucrose (10) have been already evidenced in the male cones [2] as well as in the other studied organ of the species [8]. Indeed, isocupressic acid and arginine have not been evidenced in these cones unlike the male cones.

In Table 2, the comparison among the compositions of the leaves, the male and half-matured female cones is reported. In addition, for the present compounds in their corresponding cones, a semi-quantitative analysis is reported. The derived values are here expressed as percentage of the amount of compound present in the total amount of the dried crude extract subjected to analysis (mg/mg) and are also compared between the two studied cones.

As it can be seen from the table, only two compounds, acetyl-isocupressic acid (1) and sandaracopimarcic acid (3) have been found in all the three organs. Indeed, six compounds have been evidenced in two organs, whereas all the remaining ones only in one organ. For what concerns the amounts of them, it was observed that for acetyl-isocupressic acid (1), sandaracopimarcic acid (3) and glucose (9), the relative percentages were much higher in the half-matured female cones. On the other hand, the relative percentage of wollemol (4) is higher in the male cones and the relative percentage of shikimic acid (7) is higher in the leaves. In addition, the relative percentages of methyl-(E)-communate (2) and sucrose (10) are quite similar between the two cones and, anyway, they were not found in the leaves.

Table 2  Presence and semi-quantitative analysis of the compounds reported in the leaves, male cones and half-matured female cones of W. nobilis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Leaves (%)</th>
<th>Male cones (%)</th>
<th>Half-matured female cones (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-isocupressic acid (1)</td>
<td>1.57%</td>
<td>1.59%</td>
<td>5.79%</td>
</tr>
<tr>
<td>Methyl-(E)-communate (2)</td>
<td>Not found</td>
<td>1.69%</td>
<td>2.68%</td>
</tr>
<tr>
<td>Sandaracopimarcic acid (3)</td>
<td>1.26%</td>
<td>1.21%</td>
<td>5.40%</td>
</tr>
<tr>
<td>Wollemol (4)</td>
<td>Not found</td>
<td>3.47%</td>
<td>1.34%</td>
</tr>
<tr>
<td>7''-O-methyl-agathisflavone (5)</td>
<td>Not found</td>
<td>Not found</td>
<td>0.68%</td>
</tr>
<tr>
<td>7-4'''-di-O-methyl-agathisflavone (6)</td>
<td>Not found</td>
<td>Not calculated</td>
<td>0.23%</td>
</tr>
<tr>
<td>Shikimic acid (7)</td>
<td>6.83%</td>
<td>1.44%</td>
<td>Not calculated</td>
</tr>
<tr>
<td>Quinic acid (8)</td>
<td>Not found</td>
<td>Not found</td>
<td>Not calculated</td>
</tr>
<tr>
<td>Glucose (9)</td>
<td>Not found</td>
<td>1.08%</td>
<td>7.96%</td>
</tr>
<tr>
<td>Sucrose (10)</td>
<td>Not found</td>
<td>1.08%</td>
<td>1.99%</td>
</tr>
<tr>
<td>Raffinose (11)</td>
<td>Not found</td>
<td>Not found</td>
<td>1.99%</td>
</tr>
<tr>
<td>D-lactic acid (12)</td>
<td>Not found</td>
<td>Not found</td>
<td>Not calculated</td>
</tr>
<tr>
<td>Succinic acid (13)</td>
<td>Not found</td>
<td>Not found</td>
<td>Not calculated</td>
</tr>
<tr>
<td>Alanine (14)</td>
<td>Not found</td>
<td>Not found</td>
<td>Not calculated</td>
</tr>
<tr>
<td>Pheophorbide a</td>
<td>Not calculated</td>
<td>Not found</td>
<td>Not found</td>
</tr>
<tr>
<td>Isocupressic acid</td>
<td>1.72%</td>
<td>1.46%</td>
<td>Not found</td>
</tr>
<tr>
<td>Agathic acid</td>
<td>0.86%</td>
<td>Not found</td>
<td>Not found</td>
</tr>
<tr>
<td>7,4''-tri-O-methyl-agathisflavone</td>
<td>0.86%</td>
<td>Not found</td>
<td>Not found</td>
</tr>
<tr>
<td>7,4''-tetra-O-methyl-agathisflavone</td>
<td>0.31%</td>
<td>Not found</td>
<td>Not found</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>1.22%</td>
<td>Not found</td>
<td>Not found</td>
</tr>
<tr>
<td>Arginine</td>
<td>Not found</td>
<td>0.36%</td>
<td>Not found</td>
</tr>
</tbody>
</table>
Yet, the even small percentages of 7''-O-methyl-agathisflavone (5) and raffinose (11) are interesting since these compounds have not been evidenced in the other organs. Lastly, shikimic acid (7), quinic acid (8), D-lactic acid (12), succinic acid (13) and alanine (14) could not be quantified due to the impossibility to calculate their relative amounts in the assembly.

Actually, the presence of all these compounds in the half-matured female cones is quite significant from the chemotaxonomic standpoint since most of them represent chemotaxonomic markers of the genus and the family. In particular, these are the diterpenes, the two biflavonoids, derivatives of agathisflavone and partially, quinic acid (8). In fact, acetyl-isocupressic acid (1), the chemotaxonomic marker of the genus, has been previously evidenced in the family only in the Wollemia genus [2, 8], whereas methyl-(E)-communate (2) and sandaracopimaric acid (3), which are chemotaxonomic markers at the family level, have been already identified in several species of the Agathis and Araucaria genera [14, 15]. The biflavonoid, derivatives of agathisflavone are considered to be chemotaxonomic markers of the family, too. Yet, 7''-O-methyl-agathisflavone (5) has been reported before only in some Agathis and Araucaria species [16-19]. Lastly, quinic acid (8) is a quite rare compound in Gymnosperms where it has been found only in two Araucaria species [20, 21].

For what concerns the other compounds, they are very common compounds in the plant kingdom thus not presenting chemotaxonomic relevance. An interesting case is given by the nor-labdane diterpene, wollemol (4), instead. This compound has been isolated for the first time in absolute in the male cones [2] and here for the second time. It is interesting to notice that it has not been found in the leaves [8] and this might indicate a particular accumulation of it right in the cones where, perhaps, the physiological conditions might favor its biosynthesis. Yet, further phytochemical studies on the same cones collected in other vegetation periods and in other growth areas are necessary in order to confirm this hypothesis.

Indeed, the presence of all these compounds is possibly important from the ethnopharmacological and nutraceutical standpoints. In fact, sandaracopimaric acid (3) exerts an antifungal activity [22]. 7''-O-methyl-agathisflavone (5) shows antiviral properties against HSV-1 and HSV-2 [23], as well as inhibiting properties on DNA topoisomerases II-α and K562 leukemia cells [24]. Shikimic acid (7) presents antiviral, anti-inflammatory, antioxidant, anticoagulant and neuroprotective effects [9]. Quinic acid (8) owns antiviral and astringent properties [25]. Glucose (9) favors brain activity and is a strong antihyperglycemic. Sucrose (10) is a good preservative and antioxidant compound [26]. Raffinose (11) is able to reduce calories in excess. D-lactic acid (12) is a food additive, a preservative and a flavoring agent. Succinic acid (13) is a good acidity regulator and excipient in food and beverage industry [27]. Lastly, alanine (14) is a dispensable amino acid. For all the remaining compounds, no pharmacological data are reported in literature. Anyway, the combination of all the above-mentioned effects may substantiate, from a phytochemical point of view, the possible positive ethnopharmacological and nutraceutical utilisations of these cones which seem to be potentially more active than the male cones according to the semi-quantitative results. In fact, higher relative quantities of beneficial compounds have been isolated together with quantities of new compounds.

Yet, two important further considerations must be taken into account. The first one is the presence of acetyl-isocupressic acid (1). In fact, this compound is known to have significant health-adverse effect causing abortion in cattle even in a late-pregnancy state [14, 28]. This situation is extremely relevant since these cones are eaten by animals and the biosynthesis of this compound may also represent a mechanism of defense from the tree to protect itself...
from herbivores. The exact mechanism of action of this compound, as well as the exact necessary doses for this effect, are still widely unknown like it is not totally sure if this compound is able to exert this effect also in humans. For this reason, there are several reserves about the real ethnopharmacological and nutraceutical value of these cones, also because the total biological activity of the phytocomplex has not been studied, yet. In this sense, more detailed pharmacological studies would be necessary to finally establish the exact properties of these cones also because higher quantities of this compound in respect with the male cones were isolated. The second factor is given by the total absence of glycerides which often participate to the general nutraceutical values of a whatever species fruit but that have not been found in this case. Actually, their absence may be totally explained by vegetative cycle factors. In fact, it is extremely plausible that these cones at the vegetative cycle of their collection did not manage to biosynthesize these compounds. Nevertheless, it’s also possible that these compounds were present in these cones and that, for some reason, they have been later re-adsorbed from the plant. Yet, also in this case, further specific ecological and biochemical studies should be performed to explain this and verify this condition.

4. Conclusions

The first phytochemical analysis ever performed on the half-matured female cones of \textit{W. nobilis} evidenced the presence of 14 compounds distributed into seven different classes of natural metabolites.

Some different compounds in respect with the male cones were showed in these cones and these proved, again, the tendency of the species to differentially accumulate the metabolites in their organs.

Moreover, the chemotaxonomic markers of the genus and of the family were also observed thus confirming the previous chemotaxonomic considerations carried out on this species.

The preliminary ethnopharmacological and nutraceutical evaluation of these cones showed a good potentiality in this sense, also supported by the semi-quantitative analysis. Nevertheless, the presence of acetyl-isocupressic acid (1), even in high amount, indicates that these cones must be further studied before giving a final positive or negative response regarding their possible utilization in these fields. Anyway, according to this work, the results seem to lead to the first direction.

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References


