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Rapid and Simultaneous Extraction of Bisabolol and Flavonoids from *Gymnosperma glutinosum* and Their Potential Use as Cosmetic Ingredients

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Abstract: *Gymnosperma glutinosum* is a plant popularly known as "popote", "tatalencho", "tezozotla" or "pegajosa", and it is used in traditional medicine in the region of Tehuacán, Puebla (Mexico), for the treatment of jiotes and acne and to cure diarrhea using the aerial parts in infusions. To analyze the phytochemical composition, we have developed a rapid protocol for the extraction and separation of the components of the aerial parts of *G. glutinosum*. After a maceration process, chloroformic and methanolic extracts were obtained and analyzed. Extracts were evaluated by GC-MS (gas chromatography-mass spectrometry), and their composition revealed the presence of (−)-α-bisabolol (BIS) as the main component in the chloroformic extract, which was isolated and analyzed by ^{1}H NMR to confirm its presence in the plant. The analysis of methanolic extracts by UPLC-MS (ultra-performance liquid chromatography-mass spectrometry) revealed the occurrence of six methoxylated flavones with *m*/*z* 405.08 ($C_{19}H_{18}O_{10}$), *m*/*z* 419.09 ($C_{20}H_{20}O_{10}$) and *m*/*z* 433.11 ($C_{21}H_{22}O_{10}$), and a group of C20-, C18-hydroxy-fatty acids, which give the plant its sticky characteristic. The presence of BIS, an important sesquiterpene with therapeutic skin effects, as well as some antioxidant compounds such as methoxylated flavones and their oils, could play an important role in cosmetology and dermatology formulations.

Keywords: *Gymnosperma glutinosum*; cosmetology; skin care; antioxidants; flavonoids; bisabolol

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1. Introduction

The human skin is an organ that covers 15% of the total weight of the human body, which is not only important for aesthetic reasons, but also because it is responsible for many vital functions, among them the protection against external factors, regulation of fluid balance, metabolism, elimination of toxins and body shape maintenance [1,2]. Most people care about maintaining healthy skin, which promotes mental health by increasing people's self-confidence [3–5]. The use of cosmetics has become essential in our society, and although many plant products have been replaced by synthetic chemical compounds, a replacement has not been found for the safety and efficacy of natural products, which is why in recent years, the preference for natural products has resurfaced [6–10]. Since the awareness of the long-term benefits of natural ingredients in cosmetic products is increasing, they are being considered more, and recent studies indicate that plant components, such as phenolics, flavonoids and polysaccharides, have a high potential for cosmetic applications [11–15]. Besides the presence of compounds that demonstrated certain benefits, it is important

to consider their synergy. Extracts from medicinal plants are rich in compounds that act synergistically, and it is important to study these compounds in their natural percentage to understand their optimal biological activity [15]. Thus, in mild skin disorders, the topical application of certain preparations based on natural products, such as infusions, creams, balms and tinctures, having in mind these concepts, can be effective in preventing the development of more severe diseases.

Ethnobotanical studies have helped us to understand the use of plants in traditional medicine [16]. These studies have not only helped us to understand the historical relevance of plants but also the importance they play in human health. Based on this type of study, it was determined that *G. glutinosum*, a plant that is popularly known as "popote", "tatalencho", "pegajosa" or "tezozotla", is used in traditional medicine in the region of Tehuacán, Puebla (Mexico) [17–21], for the treatment of jiotes and acne and in infusions to cure diarrhea. In recent years, ethnobotanical studies showed that *G. glutinousm* is one of the most important plants used in traditional medicine from the Tehuacan-Cuicatlan Biosphere Reserve, San Rafael, Coxcatlan, and Zapotitlan Salinas, Puebla (Mexico), for the treatment of diarrhea. Phytochemical studies of *G. glutinosum* have demonstrated the presence of essential oils, flavonoids and diterpenes. Most of the compounds isolated from *G. glutinosum* are methoxylated flavones such as 5,7-dihydroxy 3,6,8-trimethoxyflavone and 5,7-dihydroxy 3,6,8,2′,4′,5′-hexamethoxyflavone, and some ent-labdane-type diterpene [20–23]. More recent studies showed the isolation of a diterpene ent-labdene-type: ent-dihydrotumanoic acid (DTA) with antidiarrheal and antinociceptive effects [24–26]. However, even when this plant is used in the treatment of some skin problems, there are no studies about the relation between the isolated compounds and these diseases.

(−)-α-bisabolol (BIS), a sesquiterpene alcohol, has been mostly isolated from chamomile [27], and there are no reports of the presence in the genus *Gymnosperma*. BIS has different biological activities, including antioxidant, anticancer, anti-inflammatory, anti-infection, and skin-shoothing and -moisturizing properties [28–31]. At present, BIS is mainly manufactured through the steam-distillation of chamomile essential oils. Some products are produced by synthesized BIS; however, the process requires an additional economically unviable purification step due to the presence of the diastereomer (+)-α-bisabolol [32]. Therefore, finding new natural sources of bisabolol is essential to specialty pharmacological and cosmetologically industries.

In this work, based on ethnobotanical studies, we studied *G. glutinosum* to find compounds as ingredients for cosmetic purposes, and three groups of compounds were identified under the bases of GC-MS and UPLC-MS analyses. (−)-α-bisabolol and 6-epi-shyobunol, six methoxylated flavones, and a group of C20-, C18-hydroxy-fatty acids were identified in this extract and represent interesting mixtures for further investigations in the cosmetic industry.

2. Materials and Methods

2.1. Chemicals

Chloroform (CAS 110-54-3, 99%), acetonitrile (CAS 75-05-8, 99%), methanol (CAS 64-578-6, 99%), water (CAS 7732-18-5) and the ESI-TOF (electrospray ionization-time of flight) tuning mix calibrant were obtained from Supelco (Toluca, Edo. de México, México). The deuterated $CDCl_3$, MeOD and TMS were acquired from Sigma Aldrich (Toluca, Edo. de Mexico, Mexico).

2.2. UPLC and GC Coupled to Mass Spectrometry Analysis

Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis was conducted in a Ultimate3000 UPLC system (Dionexcorp., Sunnyvale, CA, USA) with photodiode array detection (PAD), coupled to a Bruker MicrOTOF-QII system by an electrospray ionization (ESI) interface (Bruker Daltonics, Billerica, USA). Mobile phase used in the system consisted of 0.1% formic acid in water (A) and acetonitrile (B) using a gradient program of 5–35% (B) in 0–10 min, 35–80% (B) in 10–10.1 min, 80–80% (B) in

10.1–11, 80–45% (B) in 11–11.1, 45–5% (B) in 11.1–12 min and 5% (B) in 12–15 min. The chromatographic column used was a Hypersil C18 column (3.0 µm, 125 × 4.0 mm) (Varian). The solvent flow rate used was 0.5 mL/min, and the column temperature was set to 30 °C. For the mass spectrometer, conditions in the negative mode were as follows: drying gas (nitrogen), flow rate, 8 L/min; gas temperature, 180 °C; scan range, 50–3000 m/z; end plate off-set voltage, −500 V; capillary voltage, 4500 V; and nebulizer pressure, 2.5 bar.

Direct injection electrospray ionization-mass spectrometry (DI-ESI-MS) analyses were conducted on Bruker MicrOTOF-QII system by an electrospray ionization interface (Bruker Daltonics, Billerica, MA, USA) operating in the negative ion mode (ESI-).

A total of 1 mg of the sample was resuspended in 1 mL of methanol, filtered through a 0.25 µm polytetrafluoroethylene (PTFE), and diluted 1:100 with methanol to avoid saturation of the capillary and cone soiling. To inject the sample directly into the spectrometer, a 74900-00-05 Cole Palmer syringe pump (Billerica, MA, USA) was used and set at 8 µL/min to obtain a constant flow rate. The capillary voltage was set to 4500 V, and nitrogen was used as a drying and nebulizing gas, using a flow rate of 4 L/min (0.4 Bar) with a gas temperature of 180 °C. The spectrometer was calibrated with an ESI-TOF tuning mix calibrant (Sigma-Aldrich, Toluca, Estado de Mexico, Mexico).

To analyze compounds' structure, a tandem mass spectrometry (MS/MS) analysis was performed using negative electrospray ionization with the appropriate mass set. According to the obtained pattern, suitable fragments were analyzed by a Bruker Compass Data Analysis 4.0 (Bruker Daltonics), which provided a list of possible elemental formulas using Generate Molecular Formula Editor, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (σ value) for increased confidence in the suggested molecular formula (Bruker Daltonics Technical Note 008, 2004). The accuracy threshold for confirmation of elemental compositions was established at 5 ppm.

Gas chromatography analysis was performed using a gas chromatograph 456-GC SCION TQ (Bruker, Biellerica MA, USA). The injector port was set at 220 °C with split 10. Separation was achieved using an RXI-5SIL (Fused silica, 30 m × 0.32 mm (ResteK) and helium at a flow rate of 1 mL/min. Column oven was programmed in the following conditions: 55 °C for 1.0 min, 55 °C to 155 °C at 20 °C/min, 155 °C for 2 min, 155 °C to 255 °C at 10 °C/min, 255 °C for 5.0 min, and finally from 255 °C to 280 °C at 10 °C/min and 280 °C for 5 min. The MS was set in TIC mode with an EI (electron ionization) of 70 eV.

2.3. NMR Spectroscopy

^{1}H nuclear magnetic resonance (^{1}H NMR) experiments of the soluble products were conducted on a Bruker Instruments ASCEND 750 spectrometer (Billerica, MA, USA). The resonance frequency was 750.12 MHz, with a typical acquisition time of 2.1845 s and a delay time of 1.0 s between successive acquisitions. The ^{1}H and ^{13}C chemical shifts are given in units of δ (ppm) relative to tetramethylsilane (TMS), where δ (TMS) belongs to 0 ppm.

3. Results

Aerial parts of *G. glutinosum* were collected in Santa Maria La Alta, a village in the municipality of Tehuacan, Puebla (Mexico) (18.60001:−97.65754) (Figure 1). A total of 800 g of the plant were collected in two different months to standardize the extraction process and determine if there could be some changes in the production of the phytochemical compounds. The first collection was completed in June and the second one in December.

Figure 1. Presence of *G. glutinosum* ("popote, pegajosa") in the Tehuacan Valley.

3.1. Isolation and Standardization of the Method of Extraction

With plants collected in June and December 2022, the next procedure was applied: 250 g of the aerial parts of the fresh plant material were ground and extracted two times with 500 mL of chloroform at room temperature. After filtration, the solvent was evaporated to obtain the chloroform extract. This procedure was completed twice for each plant material collected. The chloroform extract was partitioned with chloroform and methanol. Two partitioned fractions were obtained: a chloroform fraction 1 ($CHCl_3$ Fc1) and methanolic fraction 1 (MeOH Fc1). The yields are shown in Table 1.

Table 1. Yields of the fractions obtained from the *G. glutinosum* chloroform extraction.

Material Collected	Yield	$CHCl_3$ Fc1	MeOH Fc1
1 June 2022 (250 g)	18.9 g (7.56%) [1]	17.1 g	1.6 g
2 December 2022 (250 g)	19.5 g (7.80%) [1]	17.6 g	1.80 g

[1] Extraction yield is the average of two processes.

To analyze if the extraction with chloroform had been exhausted, the residue of the plant material was subject to another extraction with methanol and the extract was subject to the same partition as those applied to the chloroform extract. Two fractions were obtained: a chloroform fraction 2 ($CHCl_3$ Fc2) and a methanolic fraction 2 (MeOH Fc2). The yields are shown in Table 2.

Table 2. Yields of the fractions obtained from the *G. glutinosum* methanolic extraction.

Material Collected	Yield	$CHCl_3$ Fc2	MeOH Fc2
1 June 2022 (250 g)	28.3 g (11.32%) [1]	5.1 g	20.4 g
2 December 2022 (250 g)	27.5 g (11.00%) [1]	7.6 g	19.8 g

[1] Extraction yield is the average of two processes.

3.2. Chemical Composition Assay by GC-MS of the $CHCl_3$ Fc1

The fraction partitioned with chloroform was analyzed through GC-MS. Samples were analyzed by triplicate, injecting 1 mL, and carried out at 1 mL-min^{-1} by ultrapure helium. Two peaks were detected as the main compounds, and the mass spectra of each molecule were compared with those in the NIST (National Institute of Standards and Technology) database software. Under these conditions, 6-epi-shyobunol and (−)-α-bisabolol (BIS) were detected at 2.5 and 97.5%, respectively (Figure 2).

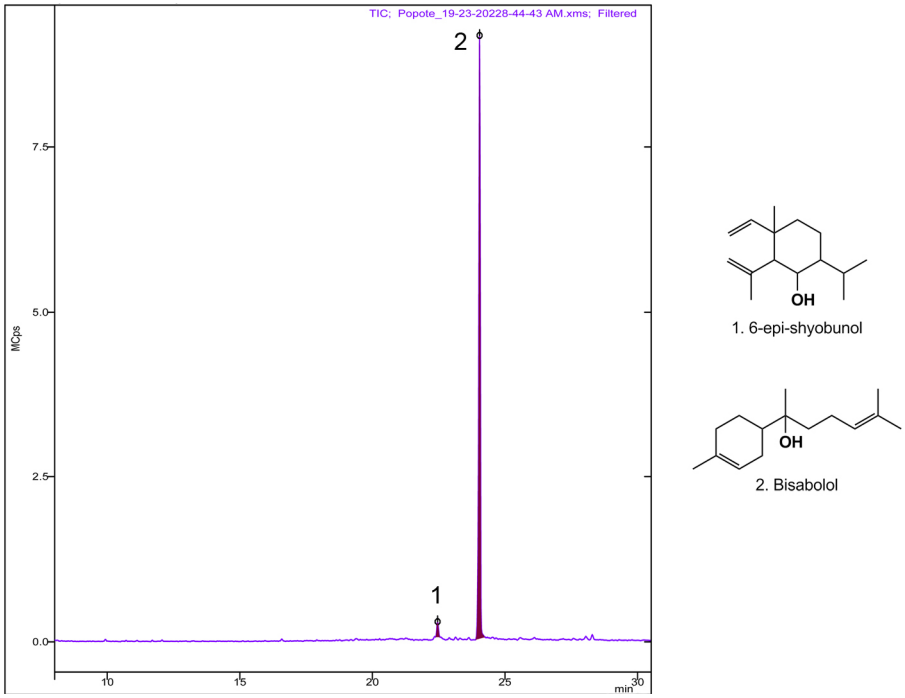

Figure 2. Chromatogram of the GC-MS analysis and compounds detected in the $CHCl_3$ Fc1 from *G. glutinosum*.

From the methanol extraction, chloroform partitioned extract ($CHCl_3$ Fc2) was analyzed under the same conditions. However, no compounds were detected indicating that BIS was completely extracted with chloroform.

3.3. Chemical Composition by UPLC-MS Assay of MeOH Fc1

MeOH Fc1 was subject to a UPLC-MS analysis and according to Figure 3, different peaks were detected and analyzed by the extracted ion chromatogram (EIC) from the total ion chromatograph (TIC) to generate two main groups of compounds detected in this extract. In the first group, two types of long-chain fatty acids were present for the m/z 321.23 and 319.21. For the detected molecular ion at m/z 321.23 $[M\text{-}H]^{-1}$, a molecular formula $C_{20}H_{34}O_3$ was assigned. According to Figure 3(1), at least six peaks correspond to this molecular weight indicating the presence of isomers. Under the bases of *ms/ms* analysis, only the main peak was identified as 20-hydroxyicosa-(5,8,11)-trienoic acid (Figure 3(1)). In the same regard, the molecular ion at m/z 319.21 $[M\text{-}H]^{-1}$ was consisting of a molecular formula $C_{18}H_{32}O_3$. In this case, four peaks were detected, indicating the presence of the same number of isomers that could be assigned to 20-hydroxyicosa-(5,8,11,14)-tetraenoic acid derivatives (Figure 3(2)).

For the second group, a methoxylated flavones group was detected and analyzed. Two peaks at Rt of 11.9 and 13.5 min (Figure 3(3)) showed the same molecular ion at m/z 405.08, which consisted of the molecular formula $C_{19}H_{18}O_{10}$ (5,7,2′,4′-Tetrahydroxy-3,6,8,5′-tetramethoxyflavone and 5,7,4′,5′-Tetrahydroxy-3,6,8,2′-tetramethoxyflavone). m/z 419.09 detected an Rt of 13.1 and 13.9 min (Figure 3(4)) and consisted of the molecular formula of $C_{20}H_{20}O_{10}$ for other two methoxylated flavones (5,7,2′-Trihydroxy-3,6,8,4′,5′-pentamethoxyflavone and 5,7,4′-Trihydroxy-3,6,8,2′,5′-pentamethoxyflavone). Finally, another two peaks at Rt of 14.3 and 15.4 min (Figure 3(5)) showed the same molecular ion at m/z 433.11, assigned to the molecular formula $C_{21}H_{22}O_{10}$ (5,7-Dihydroxy-3,6,8,3′,4′,5′-hexamethoxyflavone and 2-(5-Hydroxy-2,3-dimethoxyphenyl)-5-hydroxy-3,6,7,8-tetramethoxy-4H-1-benzopyran-4-one).

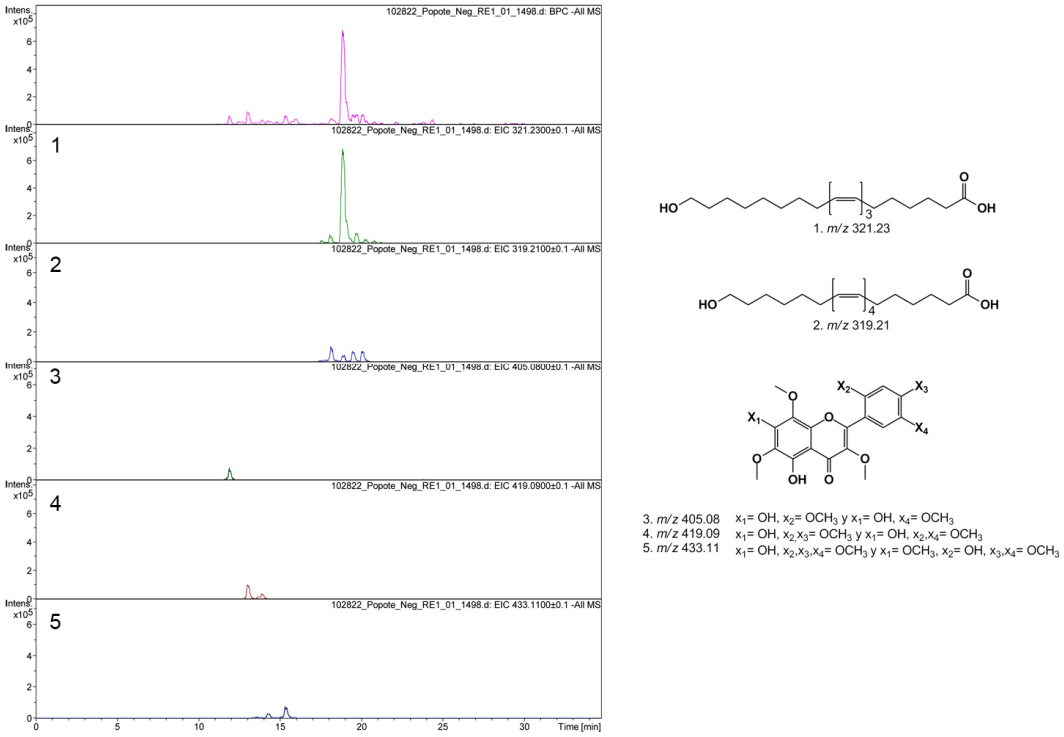

Figure 3. UPLC-MS chromatogram (upper chromatogram, TIC) and EIC-chromatogram analysis of the compounds detected in MeOH Fc1 from *G. glutinosum*. (1. EIC *m*/*z* 321, 2. EIC *m*/*z* 319, 3. *m*/*z* 405, 4. EIC *m*/*z* 419 and 5. EIC *m*/*z* 433).

The retention time (Rt), formula, name and relative percentage of the compounds detected are given in Table 3.

Table 3. Retention time and relative percentage of the compounds detected in the chromatogram of the UPLC-MS analysis of the MeOH Fc1 of *G. glutinosum*.

Rt	Formula	$[M-H]^-$ $MW_{detected}$	$[M-H]^-$ MW_{exact}	% Relative
11.9	$C_{19}H_{18}O_{10}$	405.0804	405.0816	7.0
13.1	$C_{20}H_{20}O_{10}$	419.0977	419.0972	11.0
13.6	$C_{20}H_{20}O_{10}$	405.0809	405.0816	1.1
13.9	$C_{20}H_{20}O_{10}$	419.0967	419.0972	4.1
14.3	$C_{21}H_{22}O_{10}$	433.1136	433.1129	3.0
15.4	$C_{21}H_{22}O_{10}$	433.1143	433.129	7.9
17.5	$C_{20}H_{32}O_3$	319.2197	319.2267	0.2
17.6	$C_{20}H_{34}O_3$	321.2393	321.2424	0.7
18.1	$C_{20}H_{32}O_3$	319.2195	319.2267	5.1
18.9	$C_{20}H_{34}O_3$	321.2399	321.2424	44.4
19.5	$C_{20}H_{32}O_3$	319.2191	319.2267	3.4
19.7	$C_{20}H_{34}O_3$	321.2394	321.2424	3.0
20.1	$C_{20}H_{32}O_3$	319.2197	319.2267	6.2
20.3	$C_{20}H_{34}O_3$	321.2395	321.2424	0.8
20.8	$C_{20}H_{34}O_3$	321.2398	321.2424	0.8

Under these conditions, BIS was not detected in the methanolic fraction.

The methanol extract, which was partitioned into a chloroform fraction ($CHCl_3$ Fc2) and a methanolic fraction (MeOH Fc2), was analyzed by means of DIESI-MS. As we can see in Figure 4, small peaks corresponding to the methoxylated flavones were detected, especially peaks with molecular ions at *m*/*z* 321, 405, and 419.

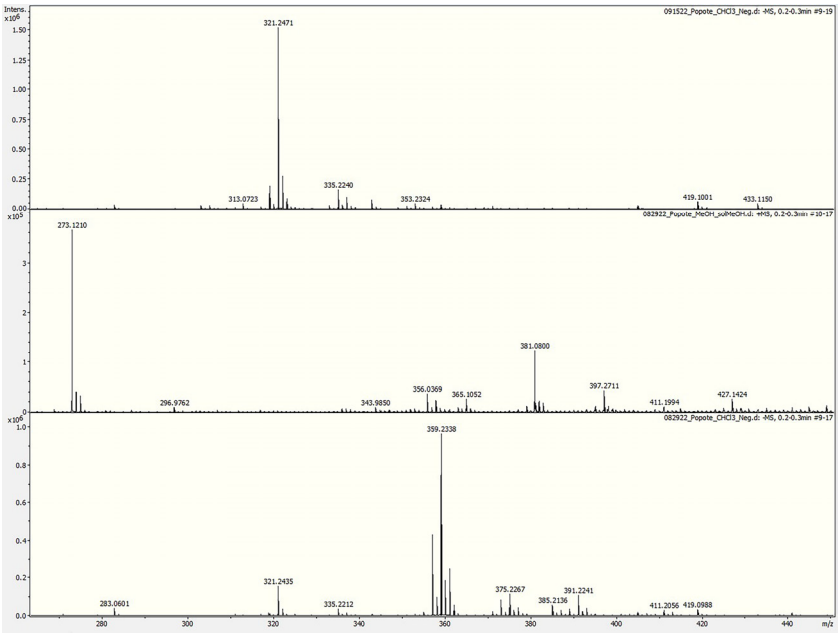

Figure 4. DIESI-MS analysis of the MeOH Fc2 from *G. glutinosum* compared with that obtained from MeOH Fc1. Upper spectrum: MeOH Fc1; middle spectrum: MeOH Fc2 and lower spectrum: $CHCl_3$ Fc2.

3.4. NMR Analysis of the Chloroform Extract of G. glutinosum

$CHCl_3$ Fc1 was analyzed through ^1H NMR. In Figure 5, a comparison of simulated and obtained spectra is shown. Vinylic protons at δ 5.2 and 5.4 ppm, as well as the methyl groups at δ 1.3 and 1.7 ppm confirm the presence of BIS, which was detected before by means of GC-MS.

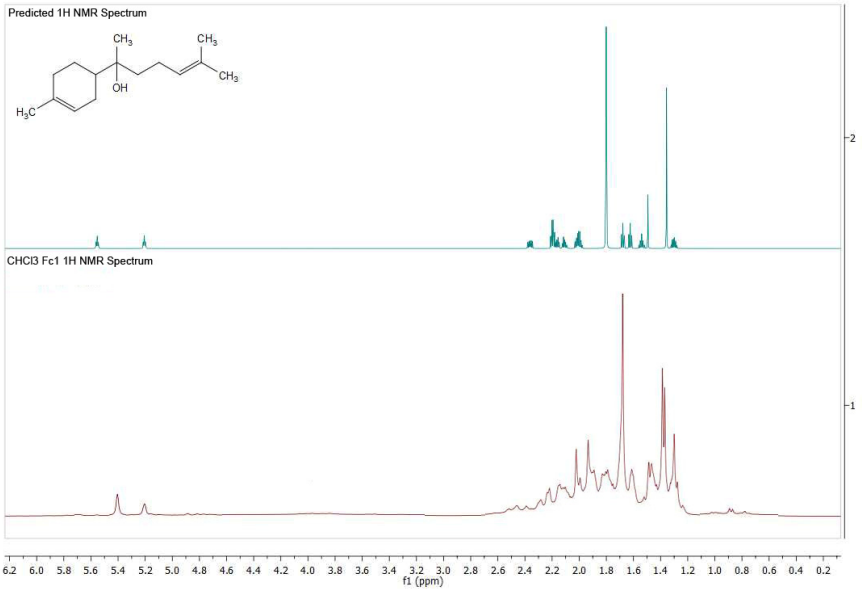

Figure 5. ^1H NMR spectra of $CHCl_3$ Fc1 from *G. glutinosum* (lower spectra), compared with a predicted spectrum of BIS (software MestReNova ver 6.0.2).

4. Discussion

In addition to the use of *G. glutinosum* in traditional medicine in the Tehuacan Valley for stomach problems, it has been used to help with some skin problems. Under these ethnobotanical studies, this plant that grows in the wild seems to have promising active

compounds, which with adequate procedures implemented for their extraction could provide a high-added value raw material source for natural antioxidant constituents with a high potential for application in cosmetology and dermatology formulations. In recent years, different studies have been conducted to incorporate plants or herbal extracts for their therapeutic potential in the market as skincare products [14,15]. In the same way, phytochemical compounds have been evaluated in vivo and in vitro models to analyze their biological activity. However, it is necessary to consider the synergy of different extracts and evaluate the activity as a complex extract because it is possible that it could have a more effective impact.

To address this objective, two extractions were performed to obtain the most compounds and characterize them under different analytical techniques.

As a plant that grows in the wild, it was important to know if the phytochemical compounds are present at any time of the year, at least in this region of México. Procedures were applied in the same way under the same conditions, and according to Tables 1 and 2, the yield in the extraction seems to be very similar.

The dry extract of chloroform was partitioned with chloroform ($CHCl_3$ Fc1) and methanol (MeOH Fc1), and $CHCl_3$ Fc1 was analyzed by GC-MS. The results obtained and previously discussed in this work suggest the presence of BIS. According to Figure 1, two peaks were identified as 6-epi-shyobunol and (−)-α-bisabolol (BIS) at 2.5 and 97.5%, respectively. To confirm this result, BIS was purified and analyzed by ^{1}H NMR. Figure 5 shows the spectra obtained and compared with a simulation of BIS in the software MestReNova. According to both analyses, the presence of this compound in *G. glutinosum* is confirmed for the first time. With the proposed methodology, BIS was extracted quickly and selectively with a good yield. So, it could be possible that the biological activity shown by this plant in skin problems can be attributed to BIS [30,31].

(−)-α-bisabolol (BIS), a monocyclic sesquiterpene alcohol, was isolated and identified for the first time from chamomile (*Matricaria chamomilla*) [27], and to date is mainly obtained from this natural source; although, it has also been isolated from the essential oil of other medicinal plants [31]. It is a compound that has been considered safe due to its low toxicity, and its effects have been widely studied in different models, indicating its potential beneficial actions [31]. BIS has a variety of biological activities, including antioxidant, gastroprotective, anti-infection and anticancer properties. In atopic dermatitis, it has been found to help attenuated pruritus and inflamed skin. Other results include improved facial texture, skin oiliness, hydrated skin, brightness and better appearance in patients who used it as a treatment [28–31].

Even though the phytochemistry of *G. glutinosum* is poorly described, and there is no report of suitable compounds that could be used as cosmetic ingredients besides BIS, some methoxylated flavones were identified. From the partitioned fraction of methanol (MeOH Fc1), a methoxylated flavones group was detected and analyzed. Two peaks at Rt of 11.9 and 13.5 min (Figure 3(3)) showed the same molecular ion at m/z 405.08, which consisted of the molecular formula $C_{19}H_{18}O_{10}$ (5,7,2′,4′-Tetrahydroxy-3,6,8,5′-tetramethoxyflavone and 5,7,4′,5′-Tetrahydroxy-3,6,8,2′-tetramethoxyflavone). m/z 419.09 detected an Rt of 13.1 and 13.9 min (Figure 3(4)) and consisted of the molecular formula of $C_{20}H_{20}O_{10}$ for two other methoxylated flavones (5,7,2′-Trihydroxy-3,6,8,4′,5′-pentamethoxyflavone and 5,7,4′-Trihydroxy-3,6,8,2′,5′-pentamethoxyflavone). Finally, another two peaks at Rt of 14.3 and 15.4 min (Figure 3(5)) showed the same molecular ion at m/z 433.11, assigned to the molecular formula $C_{21}H_{22}O_{10}$ (5,7-Dihydroxy-3,6,8,3′,4′,5′-hexamethoxyflavone and 2-(5-Hydroxy-2,3-dimethoxyphenyl)-5-hydroxy-3,6,7,8-tetramethoxy-4H-1-benzopyran-4-one).

Most of these compounds have been previously reported in *G. glutinocum*. 5,7-dihydroxy-3,6,8,2′,4′,5-hexamethoxyflavone was isolated with (−)-17-hydroxy-neo-clerod-3-en-15-oic acid by Canales et al. from two samples of *G. glutinosum* obtained in Puebla and Hidalgo State (Mexico) [20,21]. Extracts obtained from these samples showed antimicrobial activity. In 2009, Serrano et al. [22] described the isolation of two methoxylated flavones, 5,7-dihydroxy-3,6,8-trimethoxyflavone and 5,7-dihydroxy-3,6,8,2′,4′,5′-hexamethoxyflavone,

which were responsible for the fungal activity against *Aspergillus niger*, *Candida albicans*, *Fusarium sporotrichum* and *Trichophyton mentagrophytes*. Some other flavonoids, such as quercitrin, quercetin, kaempferol, rutin and vitexin have been described from *G. glutinosum* [23]. All these compounds have demonstrated their biological activity as antifungal, antimicrobial and antioxidant compounds and these activities contribute to the medicinal properties, which are used in traditional medicine in the Tehuacan, Puebla (Mexico) region. More recently, a diterpene ent-labdene-type: ent-dihydrotumanoic acid (DTA) with antidiarrheal and antinociceptive effects was isolated.

The increase in knowledge of the damage that ultraviolet radiation can cause in carcinogenesis and aging has increased the use of skin care products, especially sunscreens. However, most of these products are made with synthetic molecules or chemical substances that cause dermal toxicity. Nowadays, different reports of the beneficial effects of plants and herbal products for the skin are available [33]. Most of the plants are rich in polyphenols, flavonoids and some other compounds with antioxidant activity that can protect the skin from the effects of ultraviolet radiation. These herbal extracts should be considered for use in herbal skincare products.

Antioxidant molecules, such as methoxylated flavones, can be used to reduce and neutralize free radicals and when combined with recognized natural compounds like BIS, could improve their biological activity, resulting in their use as potential extracts implemented in cosmetic, pharmaceutical and therapeutic formulations [15,33].

One of the important characteristics of this plant is its sticky property, which is why it receives the same name in many places: "planta pegajosa (sticky plant)". This characteristic could be related to the presence of oils, waxes or fatty acids. According to the UPLC-MS analysis, two families of hydroxy-C18 and -C20 fatty acids were identified. For the detected molecular ion at m/z 321.23 $[M\text{-}H]^{-1}$, a molecular formula $C_{20}H_{34}O_3$ was assigned, and at least six peaks correspond to this molecular weight indicating the presence of isomers of the identified 20-hydroxyicosa-(5,8,11)-trienoic acid (Figure 3(1)). In the same regard, for the molecular ion at m/z 319.21 $[M\text{-}H]^{-1}$, four peaks were detected, indicating the presence of the same number of isomers that could be assigned to 20-hydroxyicosa-(5,8,11,14)-tetraenoic acid derivatives (Figure 3(2)).

The long-chain fatty acids present in the leaves of *G. glutinosum*, which are described for the first time in this work, could give the extract its waxy characteristic. Compounds such as waxes and oils extracted from plants have attracted attention for their properties to form films that can be used in cosmetic masks for skincare [34]. In this case, the presence of hydroxy long-chain fatty acids could help to keep the methoxylated flavones and the BIS in an aliphatic matrix for easier interaction with the skin. Anyway, functional groups such as hydroxyl and carbonyl groups in the long-chain fatty acids gave tunable properties to incorporate bioactive compounds for potentially sustainable alternatives over conventional products.

So, the presence of BIS, the antioxidant, the bacterial properties of the methoxylated flavones, and the aliphatic long-chain fatty acids are interesting mixtures for further investigation in the cosmetic industry of this extract obtained from *G. glutinosum*.

5. Conclusions

The use of cosmetics has become indispensable in our society. Although many plant products have been replaced by synthetic chemical compounds, the safety and efficacy of natural products have not been replaced, and in recent years, the preference for the natural has re-emerged. From an ethnobotanical study, a rapid and successful extraction of bio-compounds from *G. glutinosum* was conducted. From the chloroform extract, two partitions were obtained and analyzed. From the partition 1 (CHCl3 Fc1), the extraction and characterization of (−)-α-bisabolol as the main compound was conducted, with 97.5% of relative abundance. From partition 2 (MeOH Fc1), two groups of hydroxy-C18 and -C20 long-chain fatty acids are described for the first time in this plant. For the C20, the molecular ion at m/z 321.23 $[M\text{-}H]^{-1}$, at least six peaks indicated the pres-

ence of isomers of the identified 20-hydroxyicosa-(5,8,11)-trienoic acid (49.7%). In the same regard, for the C18, the molecular ion at m/z 319.21 $[M\text{-}H]^{-1}$, four peaks were assigned to 20-hydroxyicosa-(5,8,11,14)-tetraenoic acid derivatives (14.9%). On the other hand, under the basis of the UPLC-MS analysis, a set of methoxylated flavones are described. Two compounds with m/z 405.08, which consisted of the molecular formula $C_{19}H_{18}O_{10}$ (5,7,2′,4′-Tetrahydroxy-3,6,8,5′-tetramethoxyflavone and 5,7,4′,5′-Tetrahydroxy-3,6,8,2′-tetramethoxyflavone) (8.1%). Two compounds with m/z 419.09 consisted of the molecular formula of $C_{20}H_{20}O_{10}$ for two other methoxylated flavones (5,7,2′-Trihydroxy-3,6,8,4′,5′-pentamethoxyflavone and 5,7,4′-Trihydroxy-3,6,8,2′,5′-pentamethoxyflavone) (15.1%). Finally, there were another two compounds with m/z 433.11, assigned to the molecular formula $C_{21}H_{22}O_{10}$ (5,7-Dihydroxy-3,6,8,3′,4′,5′-hexamethoxyflavone and 2-(5-Hydroxy-2,3-dimethoxyphenyl)-5-hydroxy-3,6,7,8-tetramethoxy-4H-1-benzopyran-4-one) (10.9%).

For those with a rapid, economical and efficient process of extraction, this extract could be eligible for further studies as ingredients for cosmetic purposes since they present a set of biocompounds with useful photoprotective, moisturizing, and skin-lightening properties.

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