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Essential Oil Composition and Antibacterial Activity of *Trixis michuacana* Lex. var. *michuacana* and *Trixis michuacana* var. *longifolia*

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Abstract: The *Trixis* genus (Asteraceae) is involved in traditional medicine from Latinoamerica. In Mexico, *Trixis michuacana* Lex. var. *michoacana* and *T. michuacana* var. *longifolia* are used in the *P'urhépecha* ethnomedicine. The analysis of their volatile compounds by gas chromatography/mass spectrometry (GC-MS) is described herein. The sesquiterpene compounds were the main constituents. *β*-Caryophyllene (10.80%), germacrene D (7.93%), *β*-elemene (7.42%), and *γ*-elemene (7.37%) highlighted from var. *michuacana*, while *β*-elemene (10.02%), *α*-copaene (9.91%), *β*-caryophyllene (8.97%), germacrene D (7.40%), and *γ*-elemene (6.89%) were the major constituents from var. *longifolia*. A systematic analysis of the chemical components provided an approach to the biosynthetic pathway for volatile metabolite production. Antimicrobial assays provided scientific support for the traditional use of the plants. *In-vitro* assays using *Escherichia coli* (ATCC 25922), *E. coli* (82MR), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *S. aureus* (23MR), *S. epidermidis* (ATCC 12228), *S. epidermidis*, *Candida albicans* (17MR), *C. glabrata*, and *C. tropicalis* were achieved. These assays revealed MIC values >2 mg/mL when observed bacterial inhibition for both *Trixis* species, except for var. *michuacana* against *S. epidermidis* ATCC 12228, which was 0.5 mg/mL. These results could partially justify the use of the studied plants in ethnomedicine.

Keywords: *Trixis michuacana*; Asteraceae; antimicrobial activity; biosynthetic approach.

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1. Introduction

Trixis is an American genus belonging to the Asteraceae family, which comprises around 36 accepted species [1,2]. Interestingly, the chemical studies aimed to reveal the metabolic preferences of these species have been scarcely boarded. In literature, the chemical research of *Trixis angustifolia* [3-5], *T. antimenorrhoea* [6,7], *T. divaricata* [8,9], *T. grisebachii* [10], *T. inula* [11], *T. praestans* [12], *T. paradoxa* [13], *T. pallida* [14], *T. vauthieri* [15-17], and *T. wrightii* revealed the presence of flavonoids and terpenoid compounds, highlighting those sesquiterpenes with trixanolide skeleton [18]. Several of these plants are involved in traditional medicine in Latinoamerica, *i.e.*, the Brazilian *T. divaricata* is used to treat inflammatory processes of the ocular conjunctiva, cutaneous wounds, and uterine hemorrhages [8]. This species is also used in Argentina as an antidote against the venom of snakes from the *Bothrops* genus [9]. In Mexico, the infusion of fresh roots or aerial parts of *T. antimenorrhoea* is used to treat ophthalmic conditions as well as in case of menorrhagia [6,7], or *T. angustifolia* whose application against rheumatism, wounds, headaches, metabolic disorders, and as an antipyretic agent is suggested [4].

Other plants from the *Trixis* genus growing in Mexico are used in current traditional medicine, and their use is included in local medicinal plant repositories managed by public universities and the government. Other plants are used and supported by oral antecedents. In this sense, *T. michuacana* Lex. var. *michoacana*, commonly named *Árnica de San Cristóbal*, is frequently used as an anti-inflammatory by native populations near Pátzcuaro and Morelia cities; in fact, this plant can be found in popular markets. For its part, *Trixis michuacana* var. *longifolia* is used as an auxiliary for treatment against female infertility and inflammation, and it is commonly known as *Teparii* by the *P'urhépecha* populations around the *P'urhépecha* plateau. However, the lack of previous scientific background about these vegetal species and approaches to justify their use is advised.

As known, the secondary metabolites from useful plants for humanity can be responsible for the conferred application to the vegetal source. Among these compounds, the essential oil mixtures highlighted and their presence in industrial products, including those from the cosmetic, fragrance [19], and food sectors, are common [20]. These chemical mixtures are constituted by terpenes or phenolic derivatives containing several motifs, including alcohol, aldehyde, ethers, esters, and ketones [21]. In many cases, a key role is covered when an essential oil mixture is used as an ingredient; for example, the antioxidant [22] or antimicrobial [23] activities are used for preserving functions. Furthermore, these natural components could be used in agroindustry as insecticides [24]. Whatever the cases, the versatility of using essential oils has also been described since their incorporation in films and coating [25] and nanoemulsions [26], among others reported.

Due to the nature of the essential oil mixtures, chemical variations, including concentration and composition, could be affected by several factors [27], including external factors related to geographical influence [28-30], and internal factors, including genetic characteristics [31]. The extraction process also influences the chemical composition of essential oil [32].

The present paper describes the chemical study of the volatile compounds mixture in these plants for the first time. The chemical characterization of the essential oil from each plant was individually achieved by GC-MS analysis. Due to several inflammation complications and hidden infertility problems that could be directly associated with microbial infections, the antimicrobial activity of each essential oil mixture was individually explored. Herein, *Escherichia coli* (ATCC 25922), *E. coli* (82MR), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *S. aureus* (23MR), *S. epidermidis* (ATCC 12228), *Staphylococcus epidermidis*, *Candida albicans* (17MR), *Candida glabrata*, and *Candida tropicalis* were included in the assays. The results revealed sesquiterpenoids as the major constituents of the essential oil of both *Trixis* species, and these volatile mixtures demonstrated significant growth inhibition for *K. pneumoniae* and *Staphylococcus* species; thus, contributions to the chemistry of the *Trixis* genus and an approaching to comprise the use of these plants are herein presented.

2. Materials and Methods

2.1. Plant Material

Wild specimens of the two *Trixis* species were collected in the Michoacán State, Mexico. *T. michuacana* Lex. var. *michuacana* was collected near km 20 of Morelia-Zacapu state road No. 15, in the Capula municipality (N 19.661753, O −101.402990) at 1920 meters above the sea level, while *T. michuacana* var. *longifolia* was gathered from the San Francisco community in the municipality of Peribán (N 19.549431, O −102.405554) at 1640 meters above the sea level. Both species grow in grasslands with a temperate sub-humid climate with summer rains and medium humidity. Samples of each plant were deposited with voucher numbers EBUM 3640 and EBUM 3656, respectively, at Herbario de la Facultad de Biología (EBUM), Universidad Michoacana de San Nicolás de Hidalgo, where Prof. Rosa Isabel Fuentes Chávez and Prof. Norma Patricia Reyes Martínez achieved the taxonomic identification. The essential oil of fresh aerial parts (leaves and flowers) of *T. michuacana* Lex. var. *michuacana* (650 g) and *T. michuacana* var. *longifolia* (680 g) were extracted by steam distillation with a Clevenger apparatus using 1 L distilled water during 2 h. After the process, *T. michuacana* Lex. var. *michuacana* yielded 1.91 g (0.30%) of yellowish oil, and *T. michuacana* var. *longifolia* yielded 1.84 g (0.27%) of yellowish oil.

2.2. Analysis of Essential Oils

The essential oils of *T. michuacana* Lex. var. *michuacana* and *T. michuacana* var. *longifolia* were individually analyzed by gas chromatography-mass spectrometry (GC-MS) using a Thermo Scientific GC TRACE 1310 EM ISQLT apparatus, operated in EI mode (70 eV), equipped with split/splitless injector (250 ºC), using a TG-SQC Thermo Scientific capillary column [15 m (length) x 0.25 mm (internal diameter), film thickness: 0.25 μm]. The temperature for the TG-SQC column was 50 °C (5 min) to 250 °C at a rate of 20 °C/min. Helium was used as a carrier gas at a 1 mL/min flow rate. The components were identified by comparing their experimental relative retention index (RRI) with those from the literature. Complementarily, the mass spectra were compared with those found in NIST MS Search 2.0. (National Institute of Standards and Technology Mass Spectral Database). Relative percentage amounts of the identified components were calculated from FID chromatograms. According to GC-MS analysis, 91% of the chemical components of *T. michuacana* Lex. var. *michuacana* were revealed in the mixture. In comparison, 61.5% of the chemical composition of *T. michuacana* var. *longifolia* was exposed.

2.3. Antifungal and Antibacterial Assays

The antifungal activity of the essential oil of *T. michuacana* Lex. var. *michuacana* and *T. michuacana* var. *longifolia* was individually assayed on *Candida glabrata*, *C. tropicalis*, and *C. albicans* 17MR. The cultures were stored at room temperature using potato dextrose agar (Bioxon, Mexico State, Mexico). The antibacterial activity assays were achieved using Gram-positive bacteria, including *Staphylococcus aureus* ATCC 29213, *S. aureus* 23MR, *S. epidermidis* ATCC 12228, and *S. epidermidis*. Gram-negative bacteria also were considered and included *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *E. coli* 82MR. The bacterial strains were stored at room temperature on Müeller Hinton agar (Bioxon, Mexico State, Mexico).

C. glabrata and *C. tropicalis* were acquired from Hospital Angeles Metropolitano, Mexico, from clinical cases, while *C. albicans* 17MR, *S. aureus* 23MR, and *E. coli* 82MR were provided by the Clinical Analysis Laboratory (CUSI), Facultad de Estudios Superiores Iztacala, UNAM. *S. epidermidis* was acquired from the Microbiology Laboratory at Facultad de Estudios Superiores Cuautitlán, UNAM. *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *S. epidermidis* ATCC 12228 were acquired from American Type Culture Collection.

The screening of antibacterial activity was individually evaluated using the agar diffusion method following the guidelines outlined by the Clinical and Laboratory Standard Institute M100 [33]. For this purpose, the bacterial culture was grown in Mueller-Hinton broth (BD DIFCO, USA), and bacterial suspensions equivalent to McFarland No. 0.5 were spread on the surface of the Mueller-Hinton agar. Subsequently, sterile 5-mm disks were placed on the agar surface, and 4 μL of essential oil samples were applied. The plates were then incubated at 37 °C for 24 h. Following the incubation period, the inhibition zones were measured in mm. Chloramphenicol (25 μg/disk, Sophia Zapopan, Jalisco, Mexico) was the positive control.

Microdilution tests were performed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values [34]. Essential oil solutions ranging from 2.0 to 0.031 mg/mL were prepared from Mueller-Hinton Broth. Each concentration (100 µL) was transferred to an Eppendorf tube, followed by the addition of 100 µL of the inoculum (10^5 CFU/mL). The Eppendorf tubes were incubated in an Incu-*Shaker* (Benchmark Scientific) at 37 °C for 24 h. After incubation, 50 μL of each treatment was transferred onto the Mueller-Hinton agar Petri dish. Subsequently, the plates were incubated at 37 °C for 24 h. Each test was performed in triplicate.

The antifungal properties of the essential oils against *Candida* yeast were examined using the agar diffusion method, following a similar protocol employed for bacterial testing. A control using Nystatin (20.6 μg/disk, Bio-Rad, France) was included in the study, and all experiments were performed in triplicate.

The susceptibility of *S. epidermidis* ATCC 12228 to the essential oil *T. michuacana* Lex. var. *michuacana* was observed, which led to its selection for evaluation of its influence on growth kinetics by using the methodology outlined by López *et al* [35]. Concentrations, including ½ MIC, MIC, and MBC, were used for the study, whereas the control group was not treated with any chemical agents. Survival rates were determined in colony-forming units (CFUs) and quantified as log10 CFU/mL.
Microsoft Excel software was used for statistical analysis, and the results are presented as the mean ± standard error.

3. Results and Discussion

3.1. Chemical Composition

The essential oil composition from *T. michuacana* Lex. var. *michuacana* and *T. michuacana* var. *longifolia* is enlisted in Table 1, and the formulas of identified constituents are shown in Figure 1. The characterized compounds in both plant species were those whose pick with relative intensity was ≥0.5 of relative intensity in their respective chromatograms (Figure S1-S4).

The chemical composition of the essential oil from *T. michoacana* Lex. var. *michuacana* revealed the presence of 24 components representing 91% of the total components of the mixture, where the main constituents possess a sesquiterpene skeleton. The major constituents included the bicyclic β-caryophyllene (**3**) (10.8%), an approved food additive (FDA, Code of Federal Regulations/ No. 21CFR172.515) and bioactive sesquiterpene [38], the monocyclic germacrene D (**14**) (7.93%), β-elemene (**4**) (7.42%), and γ-elemene (**12**) (7.37%) which are appreciated by their aroma properties and potential anticancer activity [39,40]. Interestingly, 64% of the overall characterized constituents possess a saturated or unsaturated hydrocarbon skeleton. In comparison, 27% of components have an oxidized grade and included elemol (**23**) (5.05%), δ-cedrol (**28**) (3.98%), ingredients widely used in cosmetics [41,42], the antinociceptive spathulenol (**24**) [43] (4.10%), germacrene D-4-ol (**26**) (3.22%) which possesses larvicidal activity [44], cytotoxic viridiflorol (**27**) [45] (1.79%), the bicyclic and unsaturated α-cadinol (**29**) (4.50%), γ-eudesmol (**30**) (1.95%), and caryophyllene oxide (**25**) (2.43%) which are commercially offered due to their chemical and biological importance.

Table 1. Chemical composition of *T. michuacana* Lex. var *michuacana* and *T. michuacana* var. *longifolia*.

	RRI	RRI range*	Compound	Composition (%)	
				T. michuacana* Lex. var. *michuacana	***T. michuacana* var. *longifolia***
1	1322	1322-1381	*δ*-Elemene	3.32	1.21
2	1335	1334-1379	*α*-Cubebene	0.86	
3	1387	1384-1430	*β*-Caryophyllene	10.80	8.97
4	1388	1372-1403	*β*-Elemene	7.42	10.02
5	1389	1360-1392	α-Copaene	2.58	9.91
6	1391	1370-1394	*β*-Cubebene	2.62	
7	1412	1409-1448	*β*-Gurjunene	3.63	
8	1423	1413-1463	Aromandendrene		2.66
9	1433	1413-1463	Allo-aromandendrene	2.78	
10	1437	1408-1446	*β*-Copaene		1.27
11	1444	1438-1451	*α*-Himachalene	0.47	0.90
12	1451	1418-1499	*γ*-Elemene	7.37	6.89
13	1452	1435-1470	*α*-Humulene	3.52	1.28
14	1467	1458-1491	Germacrene D	7.93	7.40
15	1468	1455-1494	*γ*-Muurolene		1.04
16	1469	1467	Isopatchoulane		0.45
17	1488	1477-1502	*α*-Muurolene	2.06	
18	1489	1454-1500	*α*-Ylangene		0.54
19	1493	1490-1521	*γ*-Cadinene	2.54	
20	1500	1498-1526	*δ*-Cadinene	4.05	3.31
21	1513	1493-1513	*β*-Curcumene	2.10	
22	1538	1527-1555	*β*-Calacorene		2.02
23	1554	1518-1555	Elemol	5.05	0.47
24	1555	1549-1580	Spathulenol	4.10	
25	1558	1549-1587	Caryophyllene oxide	2.43	
26	1564	1553-1579	Germacrene D-4-ol	3.22	
27	1580	1576-1615	Viridiflorol	1.79	
28	1602	1576-1615	*δ*-Cedrol	3.98	
29	1618	1612-1642	*α*-Cadinol	4.50	3.19
30	1634	1623-1643	*γ*-Eudesmol	1.95	

*Literature reference [36] for compounds **1**-**14** and **16**-**30**, while reference [37] for compound **15**.

The chemical composition of *T. michuacana* var. *longifolia* was identified at 61.5% (Table 1). Herein, sesquiterpene compounds also resulted as the main constituents of the mixture. The major components were *β*-elemene (**4**) (10.02%), the pro-antioxidant *α*-copaene (**5**) [46] (9.91%), *β*-caryophyllene (**3**) (8.97%), germacrene D (**14**) (7.4%), and *γ*-elemene (**12**) (6.89%), representing 43% of the overall chemical constitution, and *α*-cadinol (**29**) (3.19%) and elemol (**23**) (0.47%) resulted in the oxidized constituents from essential oil. These results revealed chemical similarity for the essential oil composition, which is expected due to the taxonomic closeness of the studied plants.

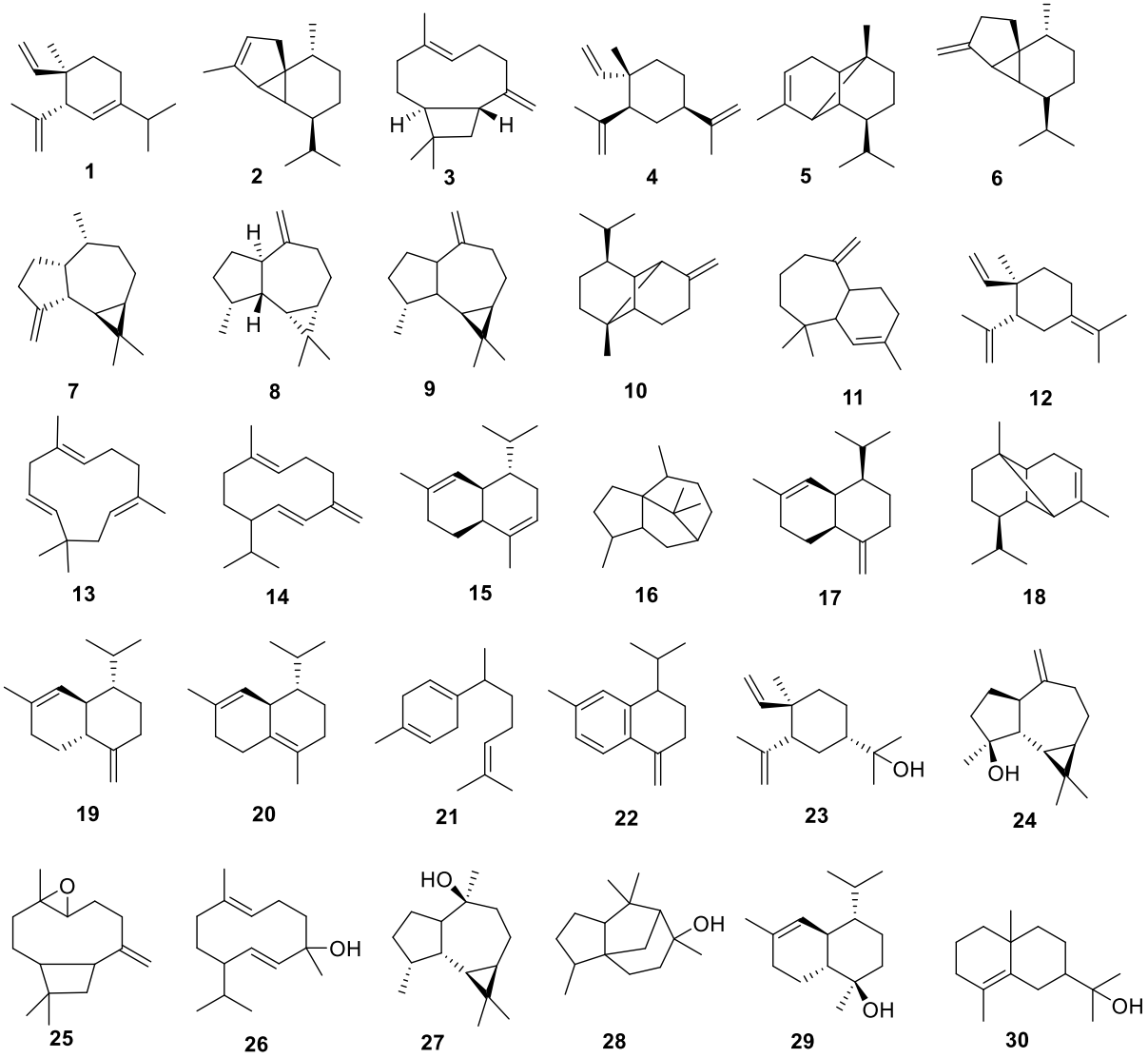

Figure 1. Formulas of essential oil constituents from *T. michuacana* Lex. var. *michuacana* and *T. michuacana* var. *longifolia*

3.2 Biogenetic Correlation of the Essential Oil Constituents

Interestingly, when the biogenetic correlation of essential oil components of *Trixis michuacana* Lex. var. *michuacana* and *T. michoacana* var. *longifolia* was achieved, an instructive panorama about the biogenesis of sesquiterpene compounds is revealed. As seen in Figure 2, once the *E,E*-farnesyl cation (**I**) is formed, a subsequent cyclization allows the *trans*-humulyl cation (**II**) or the *E,E*-germacradienyl cation (**III**). If the intermediate **II** is deprotonated, *α*-humulene (**13**) is formed, or if **II** is cyclized, *β*-caryophyllene (**3**) is yielded through the caryophyl cation (**IV**). Further oxidation processes could yield caryophyllene oxide (**25**) [47]. When the biosynthetic cascade follows cation **III**, deprotonation can occur to give germacrene A and germacrene B intermediates (not detected experimentally), whose Cope rearrangement yields *β*-elemene (**4**) and *γ*-elemene (**12**), respectively [48]. Oxidation of **12** could yield elemol (**23**). The formation of **23** from germacradienol (an oxidized derivative from **III**) after the Cope rearrangement processes could also be considered [49]. Alternatively, a 2,6-cyclization from germacradienol yields intermediary **V**, whose chemical stabilization provides *γ*-Eudesmol (**30**). For its part, the biogenesis of germacrene C and D (**14**) intermediaries is possible by the chemical stabilization of cation **IIIa**, an isomeric structure of **III**.

Cope rearrangement from germacrene C yields *δ*-Elemene (**1**) [50]. It is described that elemene-type derivatives are formed by thermal degradation from germacrene skeletons possessing *E*,*E*-1,5-diene configuration, *i.e.*, during GC-MS analysis [51,52]; consequently, germacrene D (**14**) is the only hydrocarbonate compound with germacrene skeleton herein detected, whose oxidation can lead to germacrene D-4-ol (**26**) through the intermediary **VI**, as detected in *T. michuacana* Lex. var. *michuacana*.

It follows that sesquiterpene **14** also can lead to an ionization (**VI**), which favors a cyclization to give the cadinyl cation (**VII**), the precursor of *γ*-muurolene (**15**), *α*-muurolene (**17**), *γ*- (**20**), *δ*-cadinene (**19**), and *α*-cadinol (**29**) [53-55]. An aromatization process from **17** or **19** yields *β*-calacorene (**22**) [56]. A 2,7-cyclization process from **VII** yields the copaenyl/ylangenyl cation (**VIII**), whose alternative deprotonation process favors the formation of the isomers *α*-copaene (**5**) and *β*-copaene (**10**) [57] or *α*-ylangene (**18**) [58]. On the other hand, the isomerization of **VII** by a 1,2-hydrure shift can lead to the formation of intermediary **VIIa** that favors a 1,6-cyclization to yield the cubebenyl cation (**IX**). If neutralization of cation **IX** occurs by endocyclic or exocyclic deprotonation, *α*-cubebene (**2**) or *β*-cubebene (**6**) yield, respectively [59].

Alternatively, the cyclization of **III** can lead to the formation of the 10-*epi*-*α*-guaiane intermediate (**X**), which, after Wagner Meerwein rearrangements (W-M), isopatchuolane (**16**) is formed [60]. Suppose cation **IIIa** suffers deprotonation at isopropyl moiety and promotes the formation of bicyclogermacrene (**XI**); then, a subsequent cyclization strives in ledene-type cation (**XII**), whose charge neutralization process can lead to allo-aromadendrene (**9**), aromadendrene (**8**), or viridiflorol (**27**) [61,62]. Furthermore, if carbocation from **XII** has migrated to the cyclopentenyl portion by 1,2-hydride shift (**XIIa**), followed by 1,3-hydride shift (**XIIb**), the formation of *β*-gurjunene (**7**) can occur [63]. Cation **XIIb** also could be stabilized with hydroxy moiety to gain spathulenol (**24**) [64]. Otherwise, compound **24** has been related to a possible epoxidation process (**XIa**) of bicyclogermacrene (**XI**), which activates a cyclization process (**XIb**), followed by a final cation neutralization (**XIc**) [62].

When the *E*,*E*-farnesyl cation (**I**) isomerizes to the nerodyl cation (**XIII**) to promote the cation **XIV**, a cyclization to yield the *cis*-humulyl (**XV**) intermediate is allowed. Subsequent Wagner-Meervein rearrangement (**XVa**) followed by 1,6-cyclization (**XVb**) strives in *α*-himachalane (**11**), as described [47]; however, if **XIV** is directed to cyclization to gain bisabolyl cation (**XVI**) for subsequent carbocation migration from C-7 to C-6, the homobisabolyl cation (**XVIa**) is favored, whose deprotonation yields *β*-curcumene (**21**) [65]. A 6,10-cyclization from **XVIa** yields acorenyl cation (**XVII**) whose subsequent 2,11-cyclization promotes the cedrenyl cation (**XVIII**), which oxidizes to *δ*-cedrol (**28**) [66].

3.3 Chemotaxonomic Approach

The essential oil analysis from *T. divaricata* is the unique precedent in literature [9] about essential oil composition in the *Trixis* genus and also described the presence of sesquiterpenoids as the major constituents, as herein found. *β*-Elemene (**4**), *β*-caryophyllene (**3**), and germacrene D (**14**) resulted as the main components; thus, the biogenesis of sesquiterpenoids seems to be preferred in the *Trixis* genus. In addition, the non-volatile trixanolide and germacrene derivatives [10-14] are included in this group of secondary metabolites and considered representative compounds from the genus; consequently, our results deeply complement these metabolic preferences. According to the major metabolites found in this research, the Germacrene and caryophyllene chemotype could be representative for the *Trixis* genus as the biogenetic correlation discussed above also supports; notwithstanding, future studies aimed at the essential oil studies on *Trixis* species will provide certainty about this chemotaxonomical approach.

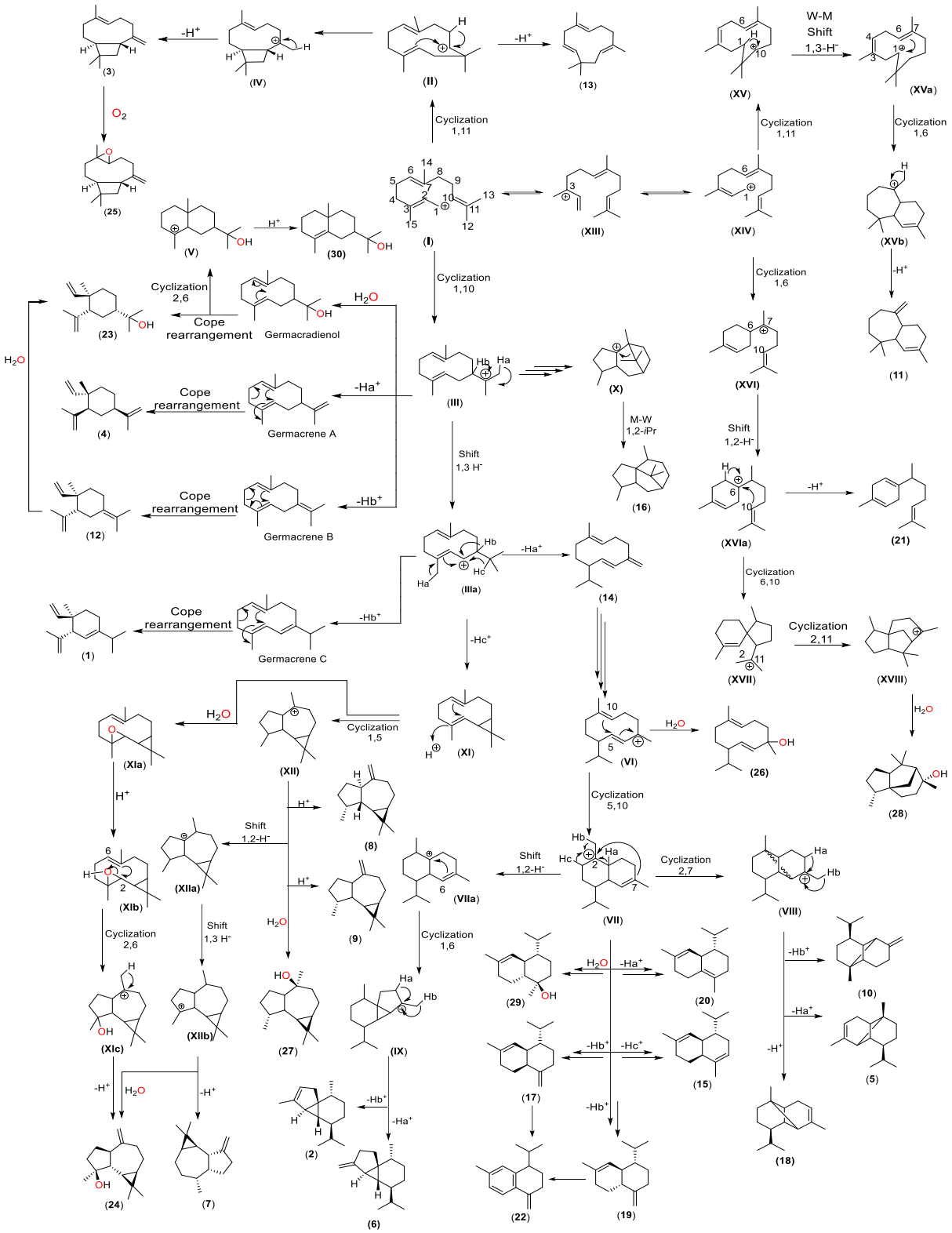

Figure 2. Proposed biogenetic pathways connecting the essential oil components from *Trixis* species

3.4 Antimicrobial Activity

In traditional medicine, *Trixis michuacana* var. *longifolia (*commonly known as *Teparii* or *Árnica de Cerro)* is considered a remedy to attend hurts, mainly in local populations around *P'urhépecha* plateau (Michoacán, Mexico). Interestingly, local healers from this location whose

medicine knowledge is inspired by the *P'urhépecha* cosmovision also suggest using this plant to promote pregnancy "*when no apparent injury that avoids such aim*". According to the information given to us firsthand by *Nana* Ilda Gonzales Buenaventura, a prominent healer from this geographical zone, this knowledge has been orally shared by generations. For its part, *Trixis michuacana* Lex. var. *michuacana* (known as *Árnica falsa*) is used for the population from Morelia city (Michoacán, Mexico) and nearby localities, including Capula, Tacícuaro, and Iratzio, to treat general inflammation, skin damage, or ear pain. This plant is commercially available in popular markets from the localities mentioned above.

Antifungal and antibacterial assays were achieved since the counteracted health problems by using the herein-studied *Trixis* plants could be associated with microbial factors. Herein, a lack of antifungal activity of the essential oil of *Trixis michuacana* var. *longifolia* against *Candida albicans* 17MR, *C. glabrata*, and *C. tropicalis* was determined. When the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were treated with this essential oil, a selective activity against *K. pneumoniae* was observed (11.30±0.58 mm). The inhibition in Gram-positive bacteria was observed, where *Staphylococcus epidermidis* resulted in the most sensible bacterial strain (21.67±0.58 mm), followed by *Staphylococcus aureus* ATCC 29213 (10.00±0.00 mm), *S. epidermidis* ATCC 12228 (9.67±0.58 mm), and *S. aureus* 23MR (7.67±0.58 mm), as enlisted in Table S1. The inhibitory effects of *T. michuacana* Lex. var. *michuacana* were observed in all assayed Gram-positive strains (see Table S1). *S. epidermidis* showed an inhibition zone of 10.67±1.15 mm, while *S. epidermidis* ATCC 12228 revealed an inhibitory zone of 8.30±0.58 mm, the same value found in *S. aureus* 23MR. Finally, *S. aureus* ATCC 29213 showed an inhibition zone of 7.67±0.58 mm. The lack of antifungal activity of the essential oil of *T. michuacana* Lex. var. *michuacana* was determined.

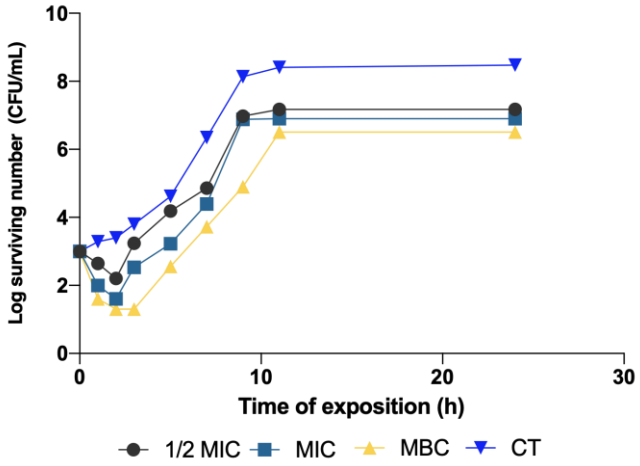

Figure 3. Growth kinetics curve of *S. epidermidis* ATCC 12228 exposed to the essential oil of *T. michuacana* Lex. var. *michuacana* at time zero, the essential oil was added to each experimental culture at concentrations of 0.250 mg/mL (½ MIC), 0.5 mg/mL (MIC), and 2 mg/mL (MBC). The control group (CT) did not receive essential oil

The MIC values were >2 mg/mL for the essential oil mixture from both *Trixis* species when bacterial inhibition was observed, except for the essential oil from *T. michuacana* Lex. var. *michuacana* against *S. epidermidis* ATCC 12228. Herein, a MIC value of 0.5 mg/mL was determined. Consequently, the bacterial survival curve for *S. epidermidis* ATCC 12228 was explored considering the ½MIC, MIC, and MBC concentrations, as shown in Figure 3. The ½MIC and MIC displayed an inhibitory effect after 2 h of exposure. In comparison, the MBC revealed an inhibitory effect for 3 h. After the period of growth inhibition, the population growth increased.

Although this research involves solely the essential oil from *T. michuacana* var. *longifolia* and *T. michuacana* Lex. var. *michuacana*, the results match with those traditional uses due to the well-known relation of *Staphylococcus* species with skin diseases as well as soft-tissue infections [67].

Furthermore, using *T. michuacana* var. *longifolia* in an aqueous hot chocolate drink is traditionally recommended, which makes sense since gained lipophilicity is appropriate to diminish essential oil volatility. According to recent studies, *S. aureus* is directly related to most deaths in individuals older than 15 years. Together with this strain, *K. pneumoniae* is included in the list of pathogens promoting mortality worldwide [68]. Moreover, *Staphylococcal* species have been associated with infertility, whose direct or through hematogenous routes can invade reproductive tissues, thus promoting undesirable health status [69].

These infectious problems have also been observed in male humans [70]. Consequently, a relevant finding for potential infection treatment explorations is described herein. Notwithstanding, several non-volatile components from the plant also should be involved in the traditionally conferred medicinal effects.

4. Conclusions

The chemical composition of two Mexican *Trixis* species is described for the first time. The presence of sesquiterpene compounds as the main components was found for the two studied species, whose biogenetic correlation revealed an instructive pathway for the biogenesis occurring in the *Trixis* genus. The antibacterial activity for the essential oil from *Trixis michuacana* Lex. var. *michuacana* and *Trixis michuacana* var. *longifolia* is herein revealed and depicted important activity against *Staphylococcus* species. The antibacterial activity against *Staphylococcus* species agreed with those ethnomedicinal uses. Chemical composition and biological activity correlated the two species herein studied. Since multi-resistant strains were used for antimicrobial assays, these essential oils resulted in potential agents for more profound antibiotic studies. Moreover, the chemical constituents from the essential oil in both studied *Trixis* species are of chemical, biological, and industrial interest, thereby further potential applications of these essential oil mixtures.

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