Research article

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Antibacterial activity and phenolic profile of the methanolic extract from the aerial parts of *Hyptis suaveolens* (Lamiaceae)

Actividad antibacteriana y perfil fenólico del extracto metanólico de las partes aéreas de *Hyptis suaveolens* (Lamiaceae)

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Abstract:

Background and Aims: *Hyptis suaveolens* is a well-known plant in Latin America for its medicinal properties. Despite its diverse uses in traditional medicine, there are few reports concerning its chemical composition. In addition, the antimicrobial activity against human-pathogen bacteria is known, but there are few reports about its activity on phytopathogenic bacteria, specifically for those affecting important crops. In this context, the main aims of this work were to determinate the antibacterial activity of *H. suaveolens* leaves on phytopathogenic bacteria, and their phenolic profile, in order to contribute to the knowledge of the phytochemical composition and bioactivity of *H. suaveolens* for phytosanitary applications.

Methods: The plant material was collected, and the aerial parts were dried, milled, and extracted with methanol. The crude extract was tested against two phytopathogenic bacterial strains (*Chryseobacterium* sp. and *Pseudomonas* sp.). Finally, phenolic compounds were identified and quantified using ultra-high performance liquid chromatography coupled with mass spectrometry.

Key results: The crude extract of *H. suaveolens* exhibited a moderate antibacterial activity against *Chryseobacterium* sp., and 14 phenolic compounds were identified and quantified, highlighting rosmarinic acid, which showed the highest concentration followed by quercetin-3-glucoside and rutin. Six phenolic compounds were identified and quantified for the first time in *H. suaveolens*.

Conclusions: In this work, the antibacterial activity of *H. suaveolens* leaves was demonstrated and it may correlate with the identification and quantification of 14 phenolic compounds, particularly with the presence of rosmarinic acid.

Key words: antimicrobial activity, mass spectrometry, phenolics.

Resumen:

Antecedentes y Objetivos: *Hyptis suaveolens* es una planta bien conocida en América Latina por sus propiedades medicinales. A pesar de sus diversos usos en la medicina tradicional, hay pocos reportes de su composición química. Además, se conoce su actividad contra bacterias que afectan al ser humano, pero existen pocos reportes acerca de su actividad sobre bacterias fitopatógenas, específicamente sobre aquellas que afectan cultivos. En este contexto los principales objetivos de este trabajo fueron determinar la actividad antibacteriana de hojas de *H. suaveolens* en bacterias fitopatógenas y su perfil fenólico, con el fin de contribuir al conocimiento de la composición fitoquímica y bioactividad de *H. suaveolens* para aplicaciones fitosanitarias.

Métodos: El material vegetal fue colectado y las partes aéreas se secaron, molieron y extrajeron con metanol. El extracto crudo fue probado contra dos cepas bacterianas fitopatógenas (*Chryseobacterium* sp. y *Pseudomonas* sp.). Finalmente, se identificaron y cuantificaron compuestos fenólicos utilizando cromatografía de líquidos de ultra alta resolución acoplada a espectrometría de masas.

Resultados clave: El extracto crudo de *H. suaveolens* mostró una actividad antibacteriana moderada en contra de *Chryseobacterium* sp., y se identificaron y cuantificaron 14 compuestos fenólicos, destacando al ácido rosmarínico, el cual mostró ser el más abundante, seguido de quercetina-3-glucósido y rutina. Seis compuestos fenólicos fueron identificados y cuantificados por primera vez en *H. suaveolens*.

Conclusiones: En este estudio se demostró la actividad antibacteriana de las hojas de *H. suaveolens* y su correlación con la identificación y cuantificación de 14 compuestos fenólicos, particularmente con la presencia del ácido rosmarínico.

Palabras clave: actividad antimicrobiana, compuestos fenólicos, espectrometría de masas.

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Introduction

The genus *Hyptis* Jacq. belongs to the family Lamiaceae and includes more than 290 species with distribution in the Neotropics (Luzuriaga-Quichimbo et al., 2018). This genus has attracted the attention of many research groups because of its biological activities, including antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer compounds (Bezerra et al., 2017; Luzuriaga-Quichimbo et al., 2018; Sharma et al., 2019; Stankovic, 2020; Mishra et al., 2021). *Hyptis suaveolens* (L.) Poit. is a perennial herb known in Mexico as donkey's herb (hierba del burro), native to tropical America but currently with pantropical distribution (Padalia et al., 2015). Studies have reported several biological activities of *H. suaveolens*, such as sedative, diuretic, antispasmodic, anti-inflammatory, among others (Mabberley, 1990; Singh et al., 1992; Mishra et al., 2021). In particular the essential oils obtained from its leaves have antimicrobial activity, finding mainly mono- and sesquiterpenes, and their oxygenated derivatives (Mishra et al., 2021). The effectiveness of extracts and essential oils of *H. suaveolens* as antibacterial has been reported, focusing on bacteria with clinical importance (Ngozi et al., 2014; Sánchez-Aguirre et al., 2020; Mishra et al., 2021), and few studies have been conducted against microorganisms of agroeconomic interest (Sharma and Tripathi, 2008; Pachkore et al., 2011). As it is known, the use of synthetic pesticides for the control of plant pathogens represents a large problem to the environment and human health, which has led to the search for safer, natural and more friendly alternatives (Oluwaseun-Adetunji et al., 2019). Most of the phytochemistry studies in polar extracts (methanolic, ethanolic and aqueous) are mainly focused on total determinations, such as total content of phenolics, flavonoids, alkaloids, and saponins, among other chemical groups (Edeoga et al., 2006; Ngozi et al., 2014; Asha et al., 2015; Azhagu-Raj et al., 2017; Sánchez-Aguirre et al., 2020; Mishra et al., 2021; Yada et al., 2021). In addition, some individual phenolic compounds have been reported, including quercetin, apigenin, chlorogenic acid and rosmarinic acid, among others (Prawatsri et al., 2013; Bezerra et al., 2017; Hsu et al., 2019; Tang et al., 2019). The lack of information about the identity of bioactive compounds present in polar extracts brought us to conceive the goals of this research, which includes the identification and quantification of phenolic compounds from a methanolic extract of *H. suaveolens* leaves and the determination of its antimicrobial activity against *Chryseobacterium* sp. and *Pseudomonas* sp., two bacteria with agroeconomic importance (San Martín-Romero et al., 2014; Martins et al., 2018).

Materials and Methods

Collection of plant material

The aerial parts of *H. suaveolens* were collected in the community La Tinaja, Veracruz, Mexico, with coordinates 19°31'00.1''N, 96°45'00.0''W in September 2014 and January 2017. A reference specimen was deposited with the voucher number Oscar Sánchez-Aguirre number 1 in the herbarium CORU "Jerzy Rzedowski Rotter" of the Faculty of Biological and Agricultural Sciences of the Universidad Veracruzana, Mexico.

Extraction process

The plant material was dried in an oven at 45 °C for 30 hours and then milled with a mortar and pestle to obtain a fine dry powder. The methanolic extract was obtained using an accelerated solvent extractor (Dionex, ASE 350, Dionex Corporation, Sunnyvale, California, USA) as previously reported (Monribot-Villanueva et al., 2019). Consequently, 21 g of the dried material was mixed with 7 g of diatomaceous earth (Thermo Scientific, Waltham, Massachusetts, USA), and placed in seven 34 ml cells (4 g/cell; Thermo Scientific, Waltham, Massachusetts, USA), then extracted with methanol (HPLC grade; Sigma-Aldrich, St. Louis, Missouri, USA), using a single cycle at 60 °C for 15 minutes. The extracts were combined, and the total volume was measured. One ml aliquots was stored at -80 °C in an ultra-low temperature freezer (Thermo Scientific, Waltham, Massachusetts, USA) for phenolic compounds determination. The solvent was evaporated under reduced pressure at 40 °C (rotatory evaporator R-II, BUCHI, Flawil, Switzerland). The methanolic extract of *H. suaveolens* leaves was evaluated in an antimicrobial assay at different concentrations from 0.125 to 21 mg/ml.

Determination of antibacterial activity

The Gram-positive strains *Chryseobacterium* sp. and *Pseudomonas* sp. were kindly supplied by Dr. Mauricio Luna Rodríguez from the Faculty of Agricultural Sciences of the Universidad Veracruzana (Xalapa, Mexico). The *Chryseobac-*

terium sp. strain was isolated from plants of *Sechium edule* (Jacq.) Sw. showing necrosis of the leaves. The fruit of *Sechium edule* (chayote; Cucurbitaceae) is a highly appreciated food in Mexico for its nutritional and functional values that include a good source of starch and vitamins, such as ascorbic and folic acids, thiamine, riboflavin and pyridoxine (San Martín-Romero et al., 2014). *Pseudomonas* sp. is a well-known phytopathogenic bacterium that infects many different crops like tobacco, potato, tomato, banana, among others, causing bacterial wilt and huge economic losses (Martins et al., 2018). The *Pseudomonas* sp. strain used in this study was isolated from damaged plants of *Luffa cylindrica* M. Roem. Both bacterial strains have been previously reported (Ramírez-Reyes et al., 2014; 2018; 2019).

Strains were kept in King agar B (Sigma-Aldrich, St. Louis, Missouri, USA) and incubated in darkness at 27 °C ± 2 °C for 24 hours in a heating oven (Binder GmbH, Tuttlingen, Germany). For the antimicrobial test, bacterial suspensions in sterile water were prepared and adjusted to a turbidity of 0.5 according to the McFarland scale (approximately 1.5 × 10^8 colony-forming units per ml). The minimum inhibitory concentrations (MICs) were determined by the microdilution method using microplates of 96 wells of 400 µl each, according to the literature (Ramírez-Reyes et al., 2019). As negative control for the antibacterial assays, 15% and 10% of dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, Missouri, USA) aqueous solution were used for *Chryseobacterium* sp. and *Pseudomonas* sp., respectively. The antibiotics oxytetracycline (Sigma-Aldrich, St. Louis, Missouri, USA) and chloramphenicol (Sigma-Aldrich, St. Louis, Missouri, USA) were used as positive controls in the antibacterial assays. The previously mentioned antibiotics were dissolved in water at concentrations of 1, 0.50, 0.25 and 0.125 mg/ml. The methanolic extract of *H. suaveolens* leaves was tested at concentrations of 21, 19, 16, 13, 10, 9, 8, 7, 6, 5, 3, 2, 1, 0.50, 0.25 and 0.125 mg/ml. Bacterial solution (10 µl), Luria-Bertani (LB; Sigma-Aldrich, St. Louis, Missouri, USA) broth (110 µl) and the treatment (80 µl) were added in each well. In the growth control treatment, only bacterial suspension (10 µl) and LB broth (190 µl) were added. The assays were performed in triplicate. The microplates were incubated at 27 ± 2 °C for 24 hours in a heating oven (Binder, Tuttlingen, Germany). The MIC of each treatment was the lowest concentration at which turbidity was not observed in the well. Afterwards, an aliquot of 10 µl of the wells without turbidity was streaked on King agar B (Sigma-Aldrich, St. Louis, Missouri, USA) and incubated at 27 ± 2 °C for 24 hours in a heating oven (Binder GmbH, Tuttlingen, Germany). The minimum bactericidal concentration (MBC) was determined as the lowest concentration that did not generate visible bacterial growth according to the literature (Ramírez-Reyes et al., 2019).

Identification and quantification of phenolic compounds

We performed a phenolics profiling to identify and quantify bioactive compounds of this chemical category. The methanolic antibacterial extract was analyzed on an ultra-high resolution liquid chromatography coupled to a triple quadrupole mass spectrometer (1290-6460, Agilent Technologies, Santa Clara, California, USA), using a dynamic multiple reaction monitoring method (dMRM) according to previous reports of our research group (Juárez-Trujillo et al., 2018; Monribot-Villanueva et al., 2019). Forty-eight phenolic compounds were searched. The compounds chlorogenic acid, caffeic acid and naringenin were determined in negative mode and their MRM transitions were 353.08>191.05, 179>135 and 271.06>151, respectively. Vanillin, quercetin-3-*O*-glucoside, rutin, kaempferol-3-*O*-glucoside, rosmarinic acid, quercetin, apigenin, kaempferol, hesperetin, nordihydroguaiaretic acid and kaempferide were determined in positive mode and their MRM transitions were 153>93, 465.2>303.04, 611.16>465.1, 449.1>287.05, 361.09>163.04, 303.05>153.1, 271.06>153.01, 287.05>153.02, 303.09>177.05, 303.16>193.12 and 301.07>258.05, respectively. For quantification, a calibration curve was constructed from 1 to 9 µM for each phenolic compound. A linear regression was applied obtaining R^2 values higher than 0.99 for each compound. Each sample was injected by triplicate.

Results

Determination of antibacterial activity

The extract only exhibited antibacterial activity against *Chryseobacterium* sp. at the highest concentration tested, determining the MIC value of 21 mg/ml (Table 1). An aliquot was streaked on King agar B (Sigma-Aldrich, St. Louis, Missouri, USA) and growing bacteria were not observed following in-

cubation, determining the MBC value as 21 mg/ml (Table 1). Unfortunately, the extract did not exhibit activity against the phytopathogenic *Pseudomonas* sp. (Table 1).

Identification and quantification of phenolic compounds

Once determined the antimicrobial activity of the methanolic extract of *H. suaveolens* leaves, we identified and quantified 14 phenolic compounds by a dMRM method using a simultaneous analysis with 48 authentic standards. The phenolic compounds identified by coelution with reference compounds were caffeic acid, vanillin, rutin, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, rosmarinic acid, quercetin, kaempferol, chlorogenic acid, naringenin, apigenin, hesperetin, nordihydroguaiaretic acid and kaempferide (Fig. 1). Rosmarinic acid exhibited the highest concentration with a concentration higher than 2700 µg of compound per gram of dried weight (DW) material (µg/g), followed by quercetin-3-*O*-glucoside and rutin with concentrations higher than 100 µg/g DW (Table 2). Caffeic acid and kaempferol-3-*O*-glucoside exhibited a content higher than 30 µg/g DW followed by quercetin, kaempferol, vanillin, chlorogenic acid, naringenin, apigenin, hesperetin, nordihydroguaiaretic acid and kaempferide with concentrations less than 20 µg/g DW (Table 2).

Discussion

Hyptis suaveolens is commonly used in the traditional medicine in different countries in America, Asia and Africa, due to its properties as sedative, diuretic, antispasmodic, anti-inflammatory, anti-catarrhal, anti-cancer and antimicrobial agent, among others (Li et al., 2020; Mishra et al., 2021). In our study, we evaluated the antimicrobial activity of the methanolic extract against two phytopathogenic bacteria that affect economical important crops. We determined that the extract only exhibited activity at the highest concentration tested against *Chryseobacterium* sp., which is known for damaging *Sechium edule* (San Martín-Romero et al., 2014). Mexico, particularly the state of Veracruz, is the main worldwide producer and exporter of *S. edule* fruit, known as chayote, and its production is severely affected by phytopathogens (Olguín-Hernández et al., 2013; San Martín-Romero et al., 2014). Chayote is commonly used as ingredient in food, but has gained recognition for its nutritional and bio-functional properties (Vieira et al., 2019; Uuh-Narváez et al., 2021). Due to the economic importance of *S. edule*, we have conducted several efforts to search for safer and natural alternatives to control the phytopathogenic *Chryseobacterium* sp. (Ramírez-Reyes et al., 2018; 2019). The antimicrobial activity against *Chryseobacterium* sp. of extracts obtained from the aerial parts of 16 plant species were previously determined (Ramírez-Reyes et al., 2018; 2019). The methanolic extracts of *Leandra cornoides* (Schltdl. & Cham.) Cogn. and *Turpinia insignis* (Kunth) Tul. and the ethyl acetate extract of *Magnolia vovidesii* A. Vázquez, Domínguez-Yescas & L. Carvajal exhibited antimicrobial activity against *Chryseobacterium* sp. (Ramírez-Reyes et al., 2018; 2019; Table 3).

The antibacterial activity against *Chryseobacterium* sp. exhibited by the methanolic extract of *H. suaveolens* (Table 1, 3) is low compared to that exhibited by the methanolic extracts of *L. cornoides* and *T. insignis* against this bacterium (Ramírez-Reyes et al., 2019; Table 3). In addition, the antimicrobial activity of the methanolic extract of *H. suaveolens* leaves is low compared to that exhibited by the ethanolic

Table 1: Antibacterial effect of extracts and antibiotics against the phytopathogens *Chryseobacterium* sp. and *Pseudomonas* sp. MIC=Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration, ND=Not determined.

Pathogenic bacteria	Treatment	MIC (mg/ml)	MBC (mg/ml)
Chryseobacterium sp.	*Hyptis suaveolens* (L.) Poit.	21	21
	Oxytetracycline	0.25	ND
	Chloramphenicol	0.50	ND
Pseudomonas sp.	*Hyptis suaveolens* (L.) Poit.	>21	ND
	Oxytetracycline	0.50	ND
	Chloramphenicol	0.50	ND

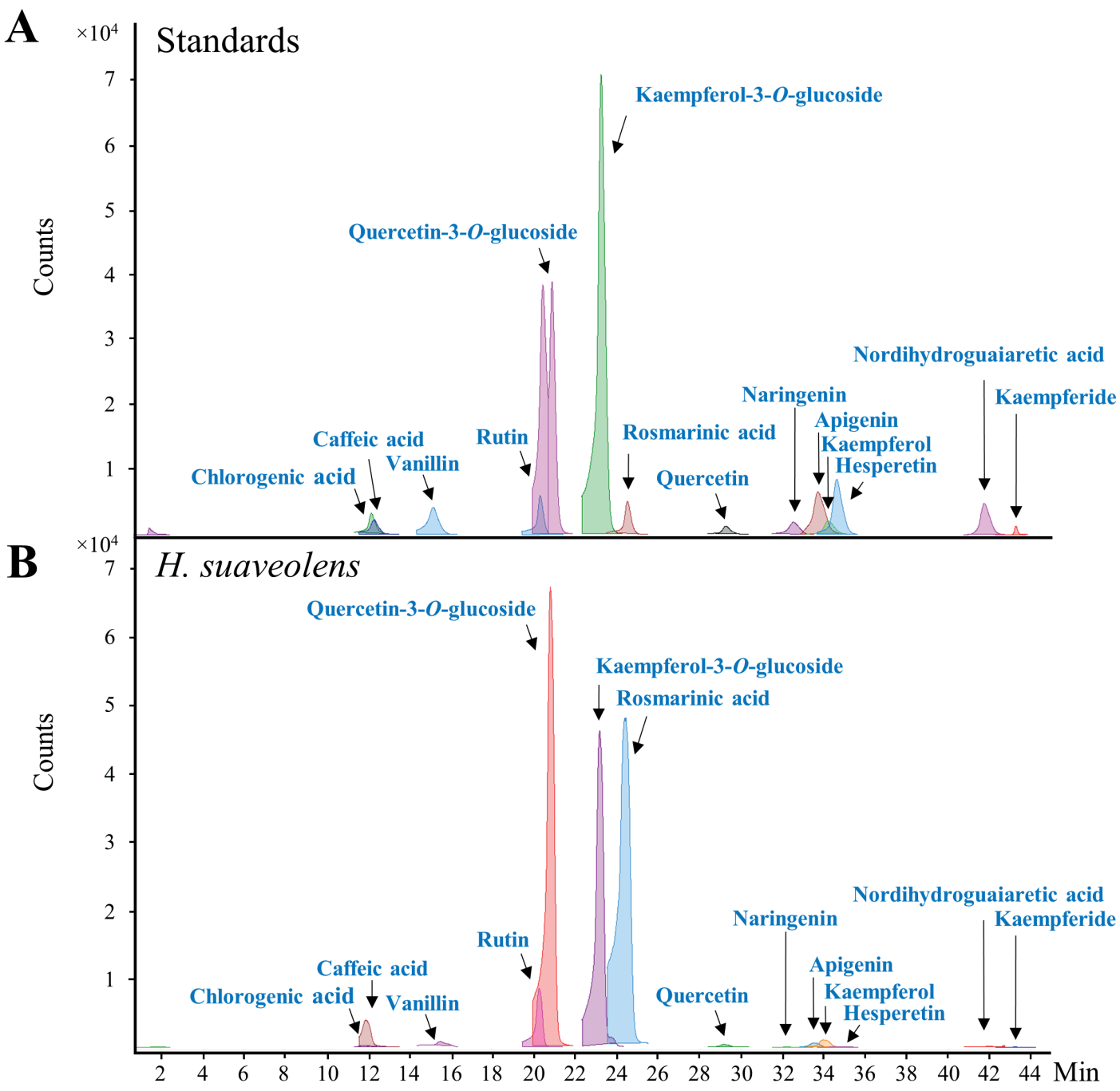

Figure 1: Representative chromatograms of: A. authentic phenolic standards mixture and B. the methanolic extract of leaves of *Hyptis suaveolens* (L.) Poit.

extract of *H. suaveolens* tested against multiple pathogenic bacteria, including *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900 in a concentration of 75-100 µg/ml (Pachkore et al., 2011; Malar et al., 2012). The hydromethanolic extract of *Hyptis atrorubens* Poit. exhibited higher antimicrobial activity against *P. aeruginosa*, inhibiting the microbial growth in a concentration of 0.3-2.5 mg/ml (Abedini et al., 2013). However, the present work is the first report of the activity of *H. suaveolens* against the phytopathogenic bacterium *Chryseobacterium* sp., which represents a new venue to explore the biological activities of this species as potential control agent in important crops.

Table 2: Phenolic compounds present in the methanolic extract from the aerial parts of *Hyptis suaveolens* (L.) Poit. Concentrations are expressed in µg of compound per gram of dried material. The average value of three replicates ± standard deviation is shown. *Concentration below the limit of quantitation.

Compound	Retention time (min)	Concentration (µg/g)
Rosmarinic acid	24.5	2736.03 ± 33.65
Quercetin-3-*O*-glucoside	20.9	226.54 ± 7.50
Rutin	20.4	159.09 ± 6.52
Caffeic acid	12.5	53.86 ± 1.47
Kaempferol-3-*O*-glucoside	23.3	34.62 ± 1.37
Kaempferol	34.2	18.03 ± 1.27
Quercetin	29.4	10.46 ± 0.84
Vanillin	15.1	2.79 ± 0.10
Chlorogenic acid*	11.9	2.94 ± 0.33
Hesperetin*	34.8	1.77 ± 0.01
Nordihydroguaiaretic acid*	41.9	1.66 ± 0.02
Apigenin*	33.4	0.62 ± 0.12
Naringenin*	32.0	0.51 ± 0.01
Kaempferide*	43.2	0.50 ± 0.01

Table 3: Antibacterial effect of extracts against *Chryseobacterium* sp. 1: Ramírez-Reyes et al., 2018; 2: Ramírez-Reyes et al., 2019; 3: this study. MIC=Minimum Inhibitory Concentration.

Species	Tissue	Solvent	MIC
Magnolia vovidesii A. Vázquez, Domínguez-Yescas & L. Carvajal[1]	Polyfollicles	Ethyl acetate	400 µg/ml
Leandra cornoides (Schltdl. & Cham.) Cogn.[2]	Leaves	Methanol	400 µg/ml
Turpinia insignis (Kunth) Tul.[2]	Leaves	Methanol	400 µg/ml
Hyptis suaveolens (L.) Poit.[3]	Leaves	Methanol	21,000 µg/ml

Regarding the identified bioactive compounds, rosmarinic acid is a well-known bioactive phenolic identified in several plant families including Lamiaceae (Petersen, 2013) and specifically in *H. suaveolens* (Prawatsri et al., 2013; Tang et al., 2019). In addition, other phenolic compounds such as simple phenolic acids, phenylpropanoids, flavonoids and tannin's precursors and derivatives have been reported in *H. suaveolens* (Prawatsri et al., 2013; Bezerra et al., 2017; Hsu et al., 2019; Tang et al., 2019). In figure 2, we summarize the information regarding the phenolic content reported in *H. suaveolens*. In our study, we identified and quantified 14 phenolic compounds in the methanolic extract of *H. suaveolens* leaves (Table 2). Besides identification, we quantified for the first time the endogenous content of rosmarinic acid in *H. suaveolens* leaves, exhibiting the highest level (Table 2). The therapeutic potential on human health for rosmarinic acid is highlighted considering the many biological activities previously reported, including anti-inflammatory, antioxidant, astringent, anti-diabetic, anti-allergic, antimutagenic, antidepressant, anti-aging, antibacterial and antiviral (Petersen, 2013; Alagawany et al., 2017; Nadeem et al., 2019; Trócsányi et al., 2020). Regarding its antimicrobial activity,

rosmarinic acid exhibited antibacterial activity against wild strains of *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872, *Micrococcus luteus* (Schroeter 1872) Cohn 1872, *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 and *P. aeruginosa* (Kuhnt et al., 1995), as well as against important human pathogen bacteria like methicillin-resistant *Staphylococcus aureus* (Rosenbach 1884), whose infections cause sepsis, toxic shock syndrome and necrotizing pneumonia (Ekambaram et al., 2016).

Along with rosmarinic acid, most of the phenolic compounds identified in *H. suaveolens* belong to the flavonoids group. We identified for the first time naringenin (Table 2), an initial precursor for the biosynthesis of downstream flavonoids such as apigenin, hesperetin, catechin, quercetin, kaempferol and their glycosylated derivatives (Fig. 2). Kaempferide, kaempferol-3-*O*-glucoside and hesperetin are being reported for the first time in *H. suaveolens* (Fig. 2, Table 2). Previous studies have shown the presence of phenolic acids like gallic, ellagic, caffeic, chlorogenic and 4-hydroxybenzoic acids in *H. suaveolens* (Bezerra et al., 2017; Hsu et al., 2019). We identified and quantified caffeic and chlorogenic acids and we are reporting for the first time vanillin, the precursor of 4-hydroxybenzoic acid (Fig. 2, Table 2). Moreover, nordihydroguaiaretic acid is a lignan that is also reported for the first time here (Table 2). Considering the global results, our study contributes to the phytochemical knowledge of *H. suaveolens*. The presence of these phenolic compounds matched with the antioxidant activity previously reported for this plant (Ngozi et al., 2014; Chigor, 2018; Sánchez-Aguirre et al., 2020) and they could be responsible in part for the antimicrobial activity observed against *Chryseobacterium* sp. (Table 1). Interestingly, in closely related species such as *H. atrorubens,* the phenolic compounds rosmarinic acid, methyl rosmarinate, hyperoside and quercetin-3-*O*-glucoside are responsible for the antimicrobial activity (Abedini et al., 2013). Rosmarinic acid and quercetin-3-*O*-glucoside

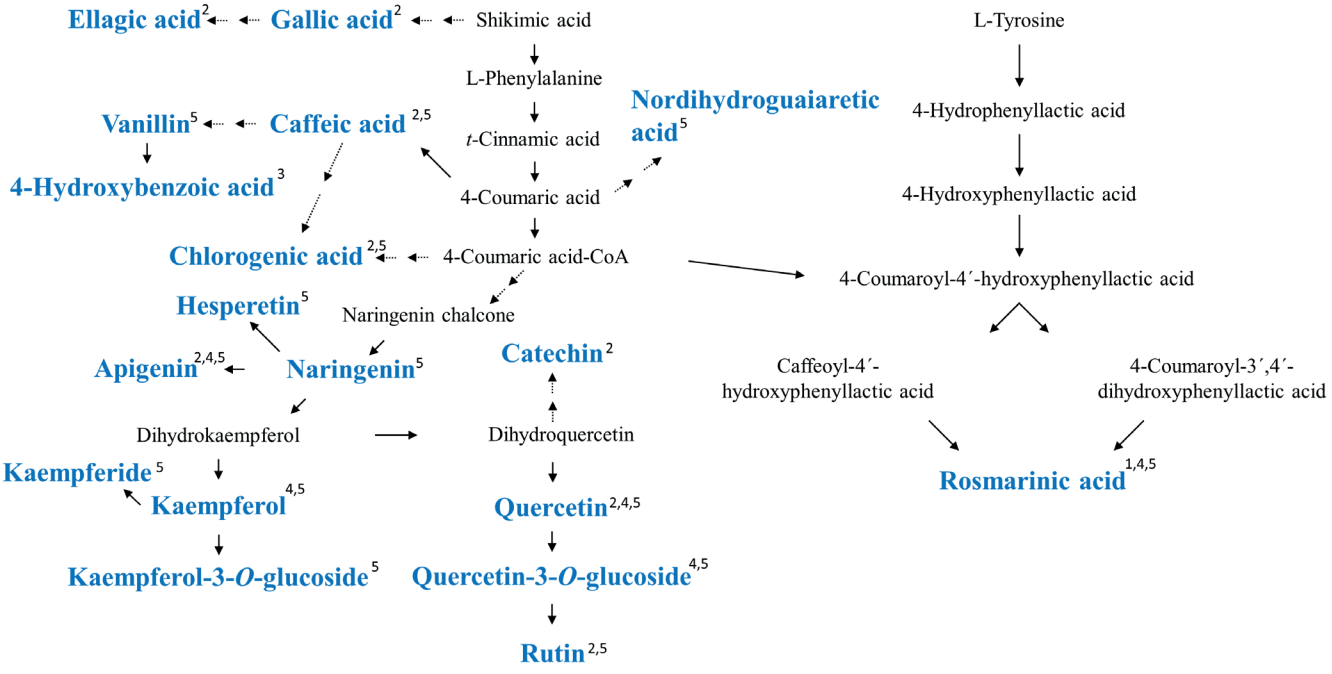

Figure 2: Reconstruction proposal of the phenolic biosynthesis pathway in *Hyptis suaveolens* (L.) Poit. based on our results and the information reported in the Kyoto Encyclopedia of Genes and Genomes database (Kanehisa et al., 2016) and in previous reports (1: Prawatsri et al., 2013; 2: Bezerra et al., 2017; 3: Hsu et al., 2019; 4: Tang et al., 2019; 5: this study; Trócsányi et al., 2020). Solid arrows indicate one-step reaction while dotted arrows indicated multi-steps reaction. The compounds identified in *H. suaveolens* are indicated in blue.

were also identified and quantified in *H. suaveolens* (Table 2). On the other hand, the antimicrobial activity against *Chryseobacterium* sp. of 12 phenolic compounds identified in the methanolic extracts of aerial parts of *L. cornoides* and *T. insignis* was previously determined (Ramírez-Reyes et al., 2019). Ellagic, gallic, gentisic, vanillic, 4-hydroxybenzoic, caffeic, ferulic, and 4-coumaric acids did not exhibit antimicrobial activity against *Chryseobacterium* sp. (Ramírez-Reyes et al., 2019). In contrast, vanillin (MIC value of 0.8 mg/ml), *t*-cinnamic acid (MIC value of 0.4 mg/ml), scopoletin (MIC value of 0.8 mg/ml), and umbelliferone (MIC value of 0.8 mg/ml) exhibited antimicrobial activity against *Chryseobacterium* sp. (Ramírez-Reyes et al., 2019). Vanillin was also identified in the methanolic extract of *H. suaveolens* at a concentration of 2.79 µg/g DW (Table 2) and could be responsible of the antimicrobial activity against *Chryseobacterium* sp. Further studies are needed to determine which other phenolic compounds contribute to the antibacterial activity displayed by the methanolic extract of *H. suaveolens*.

Conclusions

The methanolic extract of the aerial parts from *Hyptis suaveolens* exhibited antibacterial activity against *Chryseobacterium* sp., an opportunist bacterium that damages crops. As a result of the search of bioactive metabolites, 14 phenolic compounds were identified and quantified, and six of them are being reported for the first time in *H. suaveolens*. Further studies are needed to evaluate the activity of identified compounds against *Chryseobacterium* sp.

Author contributions

JAGA and MGC conceived and designed the study. JMCL, IBL, and FRF developed the experimental work in the lab. FRF performed the taxonomic identification. JLMV performed the acquisition and interpretation of mass spectrometry data. EMCM contributed to the analysis and interpretation of the antimicrobial assays. IBL, JAGA and JLMV wrote the manuscript. All authors contributed to the discussion and revision of the final version of the manuscript.

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