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Antibacterial activity of organic extracts from *Solidago graminifolia* leaves

Actividad antibacteriana de extractos orgánicos de hojas de *Solidago graminifolia*

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ABSTRACT

Solidago graminifolia (syn. *Euthamia graminifolia* (L.) Nutt) is a native species plant from North America, with abundant flavonoids, diterpenes, and polyacetylenes metabolites, that have shown cholinesterase enzyme inhibitory activity and antimicrobial activity. The aim of this study was to determine the antibacterial activity of *Solidago graminifolia* leaf extracts obtained with ethanol, dichloromethane, and hexane solvents. The *S. graminifolia* extracts were tested against *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The chemical composition of each extract was analyzed by UPLC-MS/MS. The extracs yields in ethanolic, dichloromethane and hexanoic solvents were 20.39 %, 18.34 %, and 5.3 %, respectively. The secondary metabolites identified were flavonoids, hyperoxide, quercetin, kaempferol, and some phenolic acids, such as chlorogenic acid and solidagoic acid derivatives. The ethanolic extract inhibited the five strains in all concentrations (15 mg/mL, 10 mg/mL, 5 mg/mL, and 2.5 mg/mL). The ethanol extract has a MIC of 2.0 mg/mL against *S. aureus* and 1.5 mg/mL for the Gram-negative bacteria *E. coli*, *S. enterica*, *P. aeruginosa*, and *K. pneumoniae*; the dichloromethane extract has MIC values of 2.5 mg/mL for Gram-negative strains and 2.0 mg/mL for *S. aureus*. This study showed that the ethanolic extract had the best antibacterial activity, and its biological activity can be attributed to its richness in polyphenolic compounds.

Keywords: plant, secondary metabolites, gram-negative.

RESUMEN

Solidago graminifolia (sin. *Euthamia graminifolia* (L.) Nutt) es una especie nativa de Norteamérica; es una especie abundante en flavonoides, diterpenos y policacetilenos, con actividad antimicrobiana e inhibitoria sobre la enzima acetilcolinesterasa. El objetivo fue determinar la actividad antibacteriana de tres extractos (etanol, diclorometano, hexano) de la planta *S. graminifolia* sobre *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* y *Klebsiella pneumoniae*. La composición química de cada extracto fue analizada mediante UPLC-MS/MS. El rendimiento en los extractos de etanol, diclorometano y hexano fue 20.39 %, 18.34 % y 5.3 %, respectivamente. Los metabolitos secundarios identificados fueron flavonoides, hiperósidos, quercetina, kaempferol y ácidos fenólicos, derivados del ácido clorogénico y solidagoico. El extracto etanólico inhibió las cinco cepas en todas las concentraciones (15 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL). El extracto etanólico tuvo una CMI de 2.0 mg/mL contra *S. aureus* y 1.5 mg/mL contra las bacterias Gram negativas *E. coli*, *S. enterica*, *P. aeruginosa* y *K. pneumoniae*; el extracto de diclorometano tuvo valores de CMI de 2.5 mg/mL para las cepas Gram negativas y 2.0 mg/mL para *S. aureus*. Este estudio mostró que el extracto etanólico tiene la mejor actividad antibacteriana y su actividad biológica puede ser atribuida a la presencia de compuestos polifenólicos.

Palabras clave: plantas, metabolitos secundarios, gran-negativa.

INTRODUCTION

Plants are an important source of secondary metabolites with a wide chemical diversity and different biological properties (Starks *et al*., 2010). However, the type and concentration of secondary metabolites vary according to the plant species, environmental conditions, stress factors, and other elements that condition their production (Isah, 2019).

In traditional medicine, the different parts of plants are used against diverse pathologies since their extracts have various biological activities, such as antiproliferative (Nkuimi *et al*., 2020), anti-inflammatory (Yoo *et al*., 2020), antiprotozoal (De Mieri *et al*., 2017), antibacterial (Wasihun *et al*., 2023), among others. Particularly, plants of the *Artemisia* genus have shown antibacterial activity against *Staphylococcus aureus* with a Minimum Inhibitory Concentration (MIC) of 3.0 mg/mL (Zhang *et al*., 2022). Aqueous extracts of *Alkanna tinctoria* leaves, and *Punica granatum* peel extracts have antibacterial activity against multidrug-resistant pathogens such as *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with a MIC values between 12.5 mg/ml and 25 mg/mL (Khan *et al*., 2017). Interestingly, *Solidago graminifolia* has an important antifungal effect against yeasts such as *Candida albicans* and *Candida parapsilosis* and an antibacterial effect against *Staphylococcus aureus*, with a MIC of 0.048–3.12 mg/mL (Toiu *et al*., 2019).

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The genus *Solidago* (Asteraceae) includes about 130 plant species worldwide. In particular, *Solidago graminifolia* (syn. *Euthamia graminifolia* (L.) Nutt) is a native species from North America, but there are no reports from the central region of Mexico. It is a perennial herbaceous plant with yellow flowers that has been described as a species abundant in flavonoids, such as quercetin, rutin, and astragalin metabolites, and terpenes, labdanum, diterpenes, and polyacetylenes obtained from extracts of the aerial part and roots of the plant (Szymura and Szymura, 2016; Móricz *et al*., 2020). Another similar species, *Solidago virgaurea* L., also has antioxidant, anti-inflammatory, analgesic, antifungal, and antiparasitic potential (Fursenco *et al*., 2020). In addition, its extracts have been associated with antimicrobial activity against the strains *Bacillus subtilis* F1276, *Bacillus subtilis* subsp. *spizizenii*, and *Aliivibrio fischeri*. The *Solidago graminifolia* extracts have been previously described with a promising antimicrobial effect on *Staphylocccus aureus* and *Candida albicans* species, these evaluations have been carried out in countries such as Romania and Poland (Kołodziej *et al*., 2011; Toiu *et al*., 2019). However, in our country it has not been carried out; therefore, it becomes necessary to know the biological capacity of the extracts in bacteria that impairs human health. Hence, the aim of this research was to evaluate the antibacterial activity of three organic extracts of *Solidago graminifolia* against strains of *Escherichia coli*, *Pseudomonas*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Salmonella enterica*, and to determine the secondary metabolites involved.

MATERIAL AND METHODS

Plant material collection

Leaves of *Solidago graminifolia* were collected from the municipality of Villa de Cos, Zacatecas, and Santo Domingo, San Luis Potosí, with the latitude coordinates 23.2592960-102.2226380. The plant material was placed in airtight bags and transferred to the Chemistry-Biochemistry laboratory of the Mante Multidisciplinary Academic Unit of the Autonomous University of Tamaulipas. The collected specimens were sent to the Institute of Ecology A.C. for genus and species identification, consulting specialized botanical literature and specialists of the Asteraceae family. The ITS region was amplified for molecular identification using the primers ITS-20F 5′-TCGCGTTGACTACGTCCCTGCC-3′ and ITS-262R 5′ -ATTCCCAAACAACCCGACTCG-3′ with the PCR reaction and sequencing conditions described by Herrera-Mayorga *et al.* (2022).

Obtention of organic extracts

The collected leaves were placed on aluminum trays for drying in an oven at 60 ˚C for 2 days. Subsequently, the leaves were manually pulverized until a small particle size was obtained. Solvents were used in a polarity gradient (ethanol, dichloromethane, and hexane) to obtain the extracts; 100 grams of pulverized leaves were placed in a 1 L flask with 500 mL of the corresponding solvent under constant stirring for seven days protected from light. Afterward, the solvents were filtered under a vacuum to eliminate plant material remains. The crude extract was obtained by placing the sample in a rotary evaporator at a temperature no higher than 40 °C.

Ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) analysis

One milligram of crude extract sample was weighed, dissolved in 1 mL of HPLC grade solvent, and filtered through a 0.45 µm syringe filter for analysis. The UPLC-MS/MS was carried out with an ACQUITY UPLC system coupled to a Waters QDA® mass detector (Milford, MA, USA) and an ACQUITY UPLC CORTECS® C18 1.6 µm column 3.0 x 100 mm in positive Ion mode. The column temperature was 40 °C, and the autosampler temperature was 15 °C. Elution was achieved with 0.1 % formic acid in water (Phase A), acetonitrile (Phase B), and 5 mM ammonium acetate (Phase C). The flow rate was 0.3 mL/min, and the injection volume was 5 µL. The composition of the solvents over time was initial A: 5 %; B: 85 %; C: 10 %, at 3.0 min increase, A: 15 %; B: 75 %; C: 10 %, changing at 10.0 min to A: 5 %; B: 85 %; C:10 %. The running time was 15.0 min.

Bacteria isolation and identification

The bacteria in this study were isolated from agricultural bean and corn fields soil samples, at the municipality of Fresnillo de González Echeverria (23°12′ N, 103° 30′W). The bacteria were identified molecularly by amplifying the 16S ribosomal gene and bidirectional sequencing (Herrera-Mayorga *et al*., 2023). Genomic DNA was extracted using the commercial Promega Wizard® Genomic kit (Promega A1120, USA) according to the protocol described by the manufacturer. The endpoint polymerase chain reaction was carried out using the primers Bac1-FW 5′-AGAGAGTTTGATCVTGGCTCAG-3′ and 16S-1400 RV 5′-GCGGGTGTGTGTACAAGGCCCG-3′ (Criollo *et al*., 2012), with a final reaction volume of 25 µL. The reaction was carried out with an amplification program that consisted of an initial denaturation cycle at 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 3 min in a Labnet MULTIGENE TM MINI thermocycler. The amplicons were visualized on 1.5 % agarose gel using PROMEGA's 100 bp molecular weight marker (Madison, Wis., USA). The quality of the PCR products was analyzed with a photodocumenter using the Alphalmager HP system.

The PCR product was purified using the ExoSAP-IT® protocol (Affymetrix, Santa Clara, CA). Subsequently, the bidirectional sequencing reaction was carried out using the conditions indicated in the Big Dye Terminator v.3.1 Cycle Sequencing Kit of the ABI 3130 system (Applied Biosystems, Foster City). The electropherogram obtained was visualized, edited, and assembled with the Chromas Lite 2.1 program (Technelysium) and SeqMan from the commercial DNASTAR suite of Lasergene 8 (Madison, WI). Finally, the assembled nucleotide sequences were compared with the NCBI nr/nt database using the BLASTn program for identification of the

selected bacteria ClustalW (homology > 99%) (Koolivand *et al*., 2019).

Evaluation of antibacterial activity

The antibacterial activity of the crude extracts was determined with the agar diffusion technique at four different concentrations: 15 mg/mL, 10 mg/mL, 5 mg/mL, and 2.5 mg/mL using chloramphenicol (50 µg/mL) as a positive control (Mojica *et al*., 2015). The preparation consisted of weighing the corresponding amount of each extract in a 1.5 mL Eppendorf tube, and then dissolved in 2 % DMSO. Once dissolved, it was placed in a vortex to stir until a homogeneous mixture was obtained, which, with the help of a micropipette, was added to the center of the sterile petri dish. The liquid agar was immediately poured, and the box was covered, mixing with rotary movements. Finally, each box was allowed to solidify. Each evaluation was done in triplicate and under sterile conditions (Ramírez and Marín, 2009).

A bacterial suspension was prepared for the inoculum in 0.85 % saline solution from a 24 h culture at 35 °C in nutrient agar. The inoculum solution was adjusted to tube number 5 McFarland using a spectrophotometer at a wavelength of 530 nm, obtaining a suspension at a concentration of 1 x 10^6 CFU/mL. From this suspension, 1 µL of each bacterial suspension was taken with a micropipette and sterile tips, and placed in the corresponding quadrant, trying not to pierce the agar and placing the drop as central as possible. After inoculating each box (except the negative or sterility control), it was incubated at 35 °C for 24 h. It is important to mention that each evaluation was done in triplicate and under sterile conditions. From the last two concentrations, dilutions were worked out for the measurement of MIC for each of the strains.

Minimum Inhibitory Concentration (MIC) determination

The MIC was determined only for the extracts with antibacterial activity (ethanol and dichloromethane). The evaluation was carried out with 5 concentrations (5.0 mg/mL, 2.5 mg/mL, 1.0 mg/mL, 0.5 mg/mL, and 0.1 mg/mL) to which the inoculum was added at 1x10^6 CFU/mL. Duplicates were worked with the same concentrations but without adding the inoculum to compare the turbidity of the medium. The corresponding sample was weighed in the tube with the highest concentration and subsequently dissolved in 2 % DMSO to prepare the samples with extract. The desired concentrations were adjusted in nutritious broth once a homogeneous mixture of the extract and solvent was obtained. The MIC was defined as the lowest concentration of the extract capable of total inhibition compared to the 100 % growth control (Da Silva *et al*., 2019).

RESULTS AND DISCUSSION

Plant identification

The plant was identified as *Solidago graminifolia* (syn. *Euthamia graminifolia* (L.) Nutt) by botanical experts, and genotypically had a sequence homology of 96 % (744/779) with the *Solidago graminifolia* MT610936.1 sequence. A specimen of the plant was identified taxonomically and deposited in the herbarium of Francisco González Medrano with the code UAT-22866. This species is native from North America, however, to our knowledge, this is the first report of its presence in Mexico in the states of San Luis Potosi and Zacatecas. Currently, the identification of plants by molecular biology is an easy and unexpensive tool that could be used as complementary to the traditional identification.

Organic extracts yield

The yield of organic extracts obtained by maceration from *S. graminifolia* leaves had a value of 20.39 % with ethanol, followed by dichloromethane with 18.34 %. The hexanoic extract had the lowest yield with 5.3 %. In general, the yields obtained could be considered low because, in previous research, Toiu *et al*. (2019) mention that the extracts from the aerial part of *S. graminifolia* with polar solvents, such as methanol and ethanol, produce a higher yield with values ranging from 28.01 % and 31.17 %, respectively. Additionally, they mention that polar solvents produce a high yield of total polyphenol content (192.69 mg/g extract) and flavonoids (151.41 mg/g extract), compared to chloroform (40.5 mg/g) and petroleum ether (121.2 mg/g) as non-polar solvents. This can be attributed to factors such as the solubility in the solvents, since in our study absolute ethanol was used and the authors worked with a 70 % ethanol ratio 1:20; as a medium polarity solvent, chloroform was used instead of dichloromethane and as a non-polar solvent petroleum ether while we used hexane. Another variant was the time and temperature of extraction which was worked by the authors at 60 °C with a time of 50 min, while our conditions were stirring at room temperature for seven days.

UPLC phytochemical analysis

The UPLC analysis of the organic extracts from *S. graminifolia* leaves allowed the detection of some previously reported secondary metabolites (Table 1). The most representative metabolites were flavonoids, among which were quercetin and kaempferol. In general, flavonoids are a group of molecules with greater abundance in plants, and this group of metabolites are considered to be of low toxicity and a high pharmacological capacity (Tafroji *et al*., 2022). Both metabolites have been described for their oxidative properties and for being involved in the growth inhibition of bacteria and other microorganisms, which have made them an alternative for the development of new drugs whose mechanism of action has been described in Gram positive and negative bacteria, such as *Micrococcus luteus* and *Escherichia coli*, where the greatest damage has been observed in the cell membrane causing rupture, activation of apoptosis and inhibition of the synthesis of nucleic acids and proteins. It has also been reported that combined, these two metabolites enhance the antibacterial activity by participating in the interruption of fatty acid biosynthesis, and of the formation of bacterial biofilms in strains of *Mycobacterium*, *Pseudomonas*

Table 1. Secondary metabolites in organic extracts identified by molecular weight of the plant *Solidago graminifolia* by UPLC-MS.
Tabla 1. Metabolitos secundarios identificados mediante sus pesos moleculares en los extractos orgánicos de la planta *Solidago graminifolia* realizado mediante UPLC-MS.

Extract	Chemical name	Chemical formula	Molecular weight	Molecular ion Detected m/z
Ethanol	Solidagoic acid G	$C_{21}H_{27}O_5$	361.20	361.13
	Quercetin 3-O-(6″-malonyl) hexoside	$C_{24}H_{22}O_{15}$	549.42	549.23
	Solidagoic acid E	$C_{20}H_{27}O_5$	347.15	347.09
	Solidagoic acid C	$C_{20}H_{26}O_4$	331.00	331.10
	Quercetin	$C_{15}H_{10}O_7$	302.23	303.19
Dichlormethane	16-Acetoxy-17-hydroxy-7,13Z-labdadien-15-oic acid	$C_{22}H_{34}O_5$	378.0	377.11
	Quercetin	$C_{15}H_{10}O_7$	302.23	303.28
	Murratin K	$C_{20}H_{27}O_7$	379.17	379.25
	Solidagoic acid B	$C_{25}H_{34}O_5$	414.53	415.18
	Quercetin 3-O-α-L-arabinopyranoside	$C_{20}H_{18}O_{11}$	434.3	437.17
	NR	---	---	102.05
Hexane	Rhein 8-b-D-Glucuronide	$C_{21}H_{16}O_{12}$	460.34	460.25
	1,7-Dihydroxyxanthone-6-O-B-D-glucopyranoside	$C_{19}H_{19}O_{10}$	407.09	407.28
	NR	---	---	389.25
	Luteolin 7-O-beta-D-Glucuronide	$C_{21}H_{17}O_{12}$	461.4	461.25
	Solidagoic acid B	$C_{25}H_{34}O_5$	414.53	415.24
	Kaempferol	$C_{15}H_{10}O_6$	288.25	288.25

NR: Not reported.

aeruginosa and *Vibrio cholerae* (Nguyen and Bhattacharya, 2022; Periferakis *et al*., 2022). Other constituents detected were phenolic acids, such as chlorogenic and solidagoic acid derivatives, this group of metabolites are produced by many plants to defend themselves against bacteria. Their mechanisms involve the alteration of physiological pathways for biofilm formation, membrane destruction, and alterations of cellular transport (Chen *et al*., 2022; Bozsó *et al*., 2024).

According to their polarity, some secondary metabolites identified in the ethanolic extract were quercetin and solidagoic acid derivatives (E, G, and H). These metabolites are highly polar, which justifies their extraction and have been identified in *S. virgaurea* and *S. gigantea* extracts, the presence of these constituents has been associated with antimicrobial activity (Starks *et al*., 2010; Jaisinghani, 2017). In the hydroalcoholic or polar extractions of the roots and leaves of the genus *Solidago*, large quantities of clerodane diterpenes such as solidagoic acids have been described; however, this group of metabolites, despite being very specific to this genus, has not been described in the phytochemical profile of *Solidago graminifolia* (Toiu *et al*., 2019). This may be due to the fact that this group of constituents are produced by the plant as a defense mechanism against high temperatures, which is an environmental factor in the region where it was sampled. The most abundant secondary metabolites in the dichloromethane and hexane extracts were coumarins, labdane diterpenes, and glycosidic derivatives. As reported by Toiu *et al*. (2019), there is a large difference between the content of extracted metabolites and the order of polarity, where substances such as ethanol achieve greater efficiency and diversity of metabolites due to their diffusion capacity and solubility, about four times greater than non-polar solvents such as petroleum ether or chloroform, however, solvents such as hexane reduce the matrix between polar compounds and make the extraction of non-polar or semi-polar compounds more efficient.

Other secondary metabolites that were identified in this work have been obtained from the aerial parts and roots of plants from the genus *Solidago* by different authors, such as acetylenes (esters of feverfew and dehydromatricaria), clerodane diterpenes (kingidiol and solidagoic acid A), labdane diterpenes (solidagenone and presolidagenones), benzyl benzoate and terpene derivatives, which have shown antibacterial activity and pharmacological interest (Toiu *et al*., 2019; Baglyas *et al*., 2022; Bozsó *et al*., 2024).

Bacteria identification and Antibacterial activity

The isolates obtained from agricultural soils were enteropathogenic strains (Table 2). The organic extracts were evaluated against the strains identified at four concentrations to determine their potential antibacterial activity (Table 3). The ethanolic extract had antibacterial activity against the five strains at every evaluated concentration. A similar result was found with the dichloromethane extract, except at the concentration of 2.5 mg/mL, which allowed the growth of four bacteria. Finally, it was not possible to determine the antibacterial activity in the hexanoic extract due to poor solubility in the aqueous solution. This finding may be associated with the type of non-polar components, such as kaempferol, one of the extract's most abundant secondary metabolites. This metabolite is described as a very hydrophobic molecule, practically insoluble in water, and whose main associated biological activity is its antioxidant nature in neurological diseases such as Parkinson's, Alzheimer's, epilepsy, depressive disorder, anxiety disorder, and others (Silva *et al*., 2021).

Minimum Inhibitory Concentration (MIC)

The MIC of the extracts against *E. coli*, *K. pneumoniae*, *S. enterica*, *S. aureus* and *P. aeruginosa*, are shown in Table 4. The ethanolic extract of *S. graminifolia* had lowest MIC value (2.0 mg/mL for *S. aureus* and 1.5 mg/mL for the Gram-negative bacteria *E. coli*, *S. enterica*, *P. aeruginosa* and *K. pneumoniae*). The MIC of the dichloromethane extract was slightly higher than the ethanolic extract in the case of four bacteria (2.5 mg/mL) and had the same MIC value for *S. aureus*.

Our biological activity results are similar to those described by Toiu *et al.* (2019). They obtained MIC values between 0.048 and 3.12 mg/mL for ethanol extracts of the *S. graminifolia* plant and values of 0.096 and 3.12 mg/mL for methanol extracts, against Gram-positive and negative bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Escherichia coli*. Our results and those of these authors suggest moderate activity against the bacterial strains evaluated since it is estimated that MIC levels around or less than 0.5 mg/mL suggest good antibacterial activity (Salvat *et al*., 2004).

Table 2. Bacterial strains isolated and molecularly identified.
Tabla 2. Cepas bacterianas aisladas e identificadas molecularmente.

Bacteria	Strain	Gram	Characteristic
Escherichia coli	LB226692	Negative	Enteropathogenic
Salmonella enterica	IITRCS06	Negative	Enteropathogenic
Staphylococcus aureus	NI	Positive	Enteropathogenic
Pseudomonas aeruginosa	M23	Negative	Water quality indicators
Klebsiella pneumoniae	YH43	Negative	Present in agricultural soils, fixing N_2

*NI: Not identified.

Table 3. Antibacterial activity of organic extracts of *S. graminifolia*.
Tabla 3. Actividad antibacteriana de los extractos orgánicos de *S. graminifolia*.

Extract	[ ] (mg/mL)	Strain				
		***E. coli* LB226692**	***S. enterica* IITRCS06**	***S. aureus* NI**	***P. aureginosa* M23**	***K. pneumoniae* YH43**
EtOH	15	-	-	-	-	-
	10	-	-	-	-	-
	5	-	-	-	-	-
	2.5	-	-	-	-	-
DCM	15	-	-	-	-	-
	10	-	-	-	-	-
	5	-	-	-	-	-
	2.5	+	+	-	+	+
Hex	15	ND	ND	ND	ND	ND
	10	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND
	2.5	ND	ND	ND	ND	ND
Control	Positive	+	+	+	+	+
	Negative	-	-	-	-	-
	Antibiotic/ Bacteria	-	-	-	-	-
	Antibiotic/ Solvent/ Bacteria	-	-	-	-	-
	Solvent	+	+	+	+	+

ND: not determined; (-): showed null growth; (+): showed growth; EtOH: ethanol; DCM: dichloromethane; Hex: hexane; [ ]: concentration.

Table 4. Minimum Inhibitory Concentration (mg/mL) of the organic extracts from *S. graminifolia*.
Tabla 4. Concentración Mínima Inhibitoria (mg/mL) de los extractos orgánicos de *S. graminifolia*.

Bacteria	Minimum Inhibitory Concentration [mg/mL]	
	Ethanol	Dichloromethane
Escherichia coli	1.5	2.5
Klebsiella pneumoniae	1.5	2.5
Salmonella enterica	1.5	2.5
Staphylococcus aureus	2.0	2.0
Pseudomonas aeruginosa	1.5	2.5

The extract with the best antibacterial activity was the ethanolic extract of *S. graminifolia* possibly due to its richness in polyphenolic compounds (Alves *et al*., 2013), where metabolites such as quercetin may exert a possible antibacterial activity with an ability to eliminate biofilm formation in *Bacillus subtilis* FB17, and *Enterococcus faecalis* MTCC2729 strains. In addition, the quercetin molecule causes suppressing adhesion expression in the strains *S. aureus* ATCC 6538 and ATCC 25923 (Yang *et al*., 2020).

Other representative secondary metabolites (ethanol and dichloromethane) in the extracts were phenolic acids such as solidagoic acid derivatives. Clerodane diterpenes identified by UPLC-MS as solidagoic acid have sparked interest in recent years due to their notable antibacterial, antifungal, antitumor, antifeedant for insects, and other biological activities (Li *et al*., 2016). Four bioactive diterpenes have been described in *S. gigantea* plant extracts: solidagoic acid E, solidagoic acid F, solidagoic acid H, and solidagoic acid I; the latter two have acted with moderate antibacterial activity against Gram-positive *Bacillus subtilis* subsp strains *spizizenii* and *Rhodococcus fascians* with IC_{50} values of 32.3-64.4 µg/mL (Baglyas *et al*., 2022).

CONCLUSIONS

This study determined that the ethanolic extract of *Solidago graminifolia* leaves presents antibacterial activity against *E. coli* LB226692, *S. enterica* IITRCS06, *S. aureus* NI, *P. aeruginosa* M23 and *K. pneumoniae* YH43 strains, with MIC values of 1.5 to 2.0. mg/mL. This biological activity can be attributed to secondary metabolites such as quercetin and clerodane diterpenes such as solidagoic acid E, G, and H, suggesting that these active metabolites may provide a starting point for developing or identifying more active compounds. Additionally, this research confirms the potential of this plant, which has been little studied in Mexico, and its high flavonoids and phenolic contents in ethanolic extracts. It also highlights the need for studies to improve extraction techniques and elucidate the mechanisms of action involved in the antibacterial activity for the creation of new pharmaceutical products.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

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