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Research Article
Chemical Profile, Antibacterial and Antiulcerative Effects of Fraction and Compounds from *Agave cupreata*

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Abstract

Background and Objective: *Agave cupreata* is an endemic plant from Guerrero, Mexico, which is used in traditional Mexican medicine and the production of mezcal. The secondary metabolites present in this plant have good antibacterial and anti-inflammatory activity. The objective of the present work was to determine the chemical profile and antibacterial and antiulcerogenic activity of the fractions and pure compounds obtained from the dichloromethane extract of *A. cupreta* leaves. **Materials and Methods:** Purification and characterization of the fractions was carried out by chromatographic methods and Gas Chromatography Mass Spectrometry (GCMS). Antibacterial activity was evaluated with bioautography and broth microdilution techniques using ATCC strains and drug-resistant clinical isolates, while the antiulcerogenic activity was evaluated using the ethanol-induced gastric ulcer technique in mice. **Results:** In the fractions, 12 compounds were identified, the most abundant being bis(6-methylheptyl) benzene-1,2-dicarboxylate (76-100%) and bis(2-ethylhexyl) benzene-1,2-dicarboxylate (95-100%). The FdAc24 and FdAc18 fractions presented MICs of 8 and 10 mg/mL, respectively, against ATCC strains and clinical isolates. Regarding the antiulcerogenic activity, the FdAc24 fraction and the semi-purified AC4 subfraction showed a protection percentage of 66.27 and 65.13%, respectively. **Conclusion:** Dichloromethanic fractions of *A. cupreata* exhibited antibacterial activity and inhibited ulcer formation. *Agave cupreata* could be a potential source of antibacterial and antiulcerative compounds to treat bacterial infections and stomach ailments.

Key words: *Agave cupreata*, phytochemical, bacterial resistance, MIC, antibacterial activity, antiulcerogenic effect

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The emergence and spread of antibiotic resistance among pathogenic bacteria have been a growing problem for public health in the recent decades of the 20th century and the beginning of the 21st century[1]. Due to this, the World Health Organization has generated a list of bacteria based on the threat and urgency of new antibiotics that are needed; in critical condition, bacteria such as *Klebsiella*, *Pseudomonas* and *Escherichia coli* are listed; that can cause serious and fatal infections[2]. Also, in a high priority category are some such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) has disseminated globally and become a leading cause of bacterial infection in both healthcare and community settings. Another bacterium with importance in the pathogenesis of human diseases is *Helicobacter pylori*, which is responsible for approximately 70% of gastric ulcers in the population[2,3]. According to Mancuso *et al.*[2], resistance to antibiotics is increasing and we are running out of therapeutic options, so it is important to search for compounds that promote the generation of new drugs that help combat this phenomenon. Products derived from nature represent an important source for the development of new drugs, it is estimated that 35% of drugs produced on the market originated directly or indirectly from natural products[4].

In Mexico, the *Agave* genus has great economic and cultural importance and Mexican herbalists have widely reported on its use in traditional medicine[5]. Several studies have reported the presence of secondary metabolites: Flavonoids, saponins, alkaloids, coumarins, essential oils, anthraquinones, alkaloids and terpenes. Each of them, according to their chemical structure, has some biological activity, including anti-inflammatory, antibacterial, antifungal, antioxidant, antiproliferative, laxative, antiparasitic and anticancer activity, among others[6-9].

Within the *Agave* genus is *Agave cupreata*, a little-studied endemic plant of the state of Guerrero; possessing antibacterial activity against drug-resistant clinical isolates and anti-inflammatory activity[10]. The present study was designed to obtain purified compounds from the dichloromethane extract of *A. cupreata*, which may have antibacterial and antiulcerogenic activity and could be possible candidates for the creation of new drugs.

MATERIALS AND METHODS

Study area: The study was carried out from August, 2020 to July, 2022 at the Microbiology Laboratory, Autonomous University of Guerrero and Southern Biomedical Research Center (CIBIS-IMSS).

Plants extract and fractions: Extract of leaves of *A. cupreata* previously obtained with solvent dichloromethane were used by Salazar-Pineda *et al.*[10]. For chromatographic fractionation, 800 mg of the extract was subjected to open column chromatography (OCC) in which the silica gel (Kieselgel 60, Merck, Germany) was packed in a glass column (100×1.5 cm), using 100% n-hexane as an eluent system and polarity was gradually increased using successive additions of acetone to obtain 92 aliquots (150 mL). Eluted fractions obtained were monitored by thin layer chromatography (TLC) using aluminum sheets of silica gel 60 (F254 Merck, Germany), visualized by Ultraviolet light to 302 and 365 nm and revealed with acid reagent (Sigma Chemical Co., St. Louis, Missouri, USA). The aliquots were combined according to characteristics observed by TLC in twenty-six fractions (FdAc1-FdAc26).

Compound isolation and identification: The fraction FdAc8 was submitted to semi-preparative TLC (Silica gel 60 F254 Merck, Germany); eluted with system hexane:acetone (70:30 v/v) and getting the semi-purified subfraction Ac3. The fraction FdAc11 was fractionated by OCC (Silica gel 70-230, Merck, Munich, Germany) and eluent system hexane:acetone (70:30 v/v), getting 31 subfractions that were pooled into 12 subfractions (SFdAc1-SFdAc12), from the subfraction SFdAc7 the pure compound Ac1 was obtained. The subfraction SFdAc5 was purified by semi-preparative TLC (175-225 µm, Merck, Munich, Germany) and the hexane: acetone system (70:30 v/v) getting the compound Ac2. The fraction FdAc18 was submitted to OCC (Silica gel clear pore 70-230), eluent hexane:acetone system (80:20 v/v); getting 185 subfractions that were pooled into 22 subfractions (RFdAc1-RFdAc22), obtaining the semipurified subfraction Ac4 from subfraction RFdAc16.

The compounds (Ac1 and Ac2) and semi-purified subfractions (Ac3 and Ac4) were identified by gas chromatography coupled with mass spectrometry. In an Agilent 6890 chromatograph, equipped with an HP-5MS capillary column (phenyl methylsiloxane, 30×0.25 mm, film thickness 0.25 µm). The oven temperature was increased from 50-230°C at a rate of 2°C/min. Helium was used as carrier gas with a flow rate of 1 mL/min. About 1 µL of the sample was injected in splitless mode. An Agilent 5973 mass spectrometer detector was used, operating at 70 eV ionization energy and a mass range of 200-600 Da. The determination of the compounds was achieved by comparing the spectra and retention index, using the NIST/EPA/NIH Mass Spectral library version 1.7a/ChemStation.

Antibacterial activity

Tested microorganisms: The following eight strains were obtained from American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 29213 and 25923), *Enterobacter cloacae* (ATCC 700323), *Salmonella dublin* (ATCC 9676), *Escherichia coli* (ATCC 25923 and 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212). Plus, nine clinical isolates of *Staphylococcus haemolyticus*, *Staphylococcus aureus* (543), *Staphylococcus hominis* (0433), *Klebsiella pneumoniae* (189) and *Escherichia coli* were obtained from General Hospital of Acapulco, Mexico. All strain was incubated at 37°C in Mueller Hinton (BD Bioxon, México). The inoculum for the assay was prepared by direct colony suspension with Mueller Hinton broth and adjusted to obtain the turbidity of 0.5 McFarland standard (1.5×10^8 UFC/mL).

Bioautographic method: The antibacterial activity of the fractions was determined by the direct bioautography technique[11]. The method consisted of developing TLC using silica gel 60 F_{254} (175-225 µm, Merck, Munich, Germany) using the hexane:acetone system (70:30 v/v). After elution, the plate was soaked with a *Staphylococcus aureus* ATCC 29213 suspension (1.5×10^8 UFC/mL) and was incubated at 37°C for 24 hrs. After the incubation time, the plate was revealed with a solution of 3-(4,5-dimethylthiazol-2-y1)-2,5 diphenyl-2H-tetrazolium-bromide (0.5 mg/mL; MTT, Merck, Darmstadt, Germany) and incubated again for 30 min at 37°C. Bacterial inhibition was observed as yellow spots on a violet-purple background.

Minimal inhibitory concentration (MIC): The MIC of fractions and compounds was performed according to that reported by Navarro-García *et al.*[12], with modifications. The fractions, subfractions and compounds were reconstituted to concentrations of 0.003125-32 and 0.003125-0.4 mg/mL in Dimethyl Sulfoxide (DMSO 99.9% of purity at 20%, Sigma-Aldrich grade culture). After, were mixed with 100 µL of Mueller Hinton broth (MH) in microplates (96-wells). Each dilution was inoculated with 3 µL (1.1×10^4 UFC) of bacterial inoculum. The microplates were incubated at 37°C for 24 hrs. After incubation, 30 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Merck, Darmstadt, Germany) was added to each well. The plates were reincubated at 37°C for 15 min. The MIC was defined as the lowest concentration of the tested substance that inhibited the visible growth of the bacterial strains. Amikacin (100 µg/mL) was used as standard antibiotics (positive control).

Antiulcerogenic activity

Animals: Female albino ICR mice (weight 25-30 g) (Harlan, Mexico City, Mexico) were used for this assay in accordance with the protocol approved by the Institutional Research Committee in compliance with the Official Mexican Regulation dating from 1999 (NOM-062-ZOO-1999). Mice were housed in three per cage and were maintained under standard laboratory conditions (22°C+2, 12 hrs light/12 hrs dark and water/food *ad libitum*). The animals were acclimatized to the housing facilities for 3 weeks before the experiments. All experiments were conducted on a minimum of three animals and requisites of observation were employed to obtain consistent data.

Antiulcerative assay: The animals were fasted for 24 hrs before the study and were divided into four groups (n = 3 each). One group served as a normal control group and 3 groups were administered with absolute ethanol (10% of body weight) to induce gastric ulcers, according to the following design:

- **Group 1 (CN):** Normal control treated with vehicle (1% Tween 20)
- **Group 2 (NEtOH):** Negative control treated with ethanol (10% by weight)
- **Group 3 (FdAc24+EtOH) and 4 (Ac4+EtOH):** Received FdAc24 (100 mg/kg) and Ac4 (10 mg/kg), respectively, plus ethanol (10% of body weight)

After another 1 hr, the mice were sacrificed by overdose of sodium pentobarbital (PiSa Agropecuaria) and cervical dislocation, the stomachs were removed, opened along the greater curvature and washed with water. The lesions were macroscopically examined and photographs of each of the stomachs. The total surface of the stomach (mm^2) was determined, as well as the number and severity of the hemorrhagic lesions per stomach; expressed as total ulcerated gastric surface (mm^2) by image analysis with ImageJ software 1.54. The ulceration index (UI) and the percentage of protection were calculated by the following formula[13,14]:

$$UI = \frac{\text{Ulcerated area}}{\text{Total area}} \times 100$$

$$\text{Protection (\%)} = \frac{\text{UI control} - \text{UI treated group}}{\text{UI control}} \times 100$$

Statistical analysis: Data are expressed as Mean±Standard Deviation. Statistical analysis was performed using a One-way Analysis of Variance (ANOVA) followed by a Dunnett's test where significance was considered with values of *p<0.001.

RESULTS

Chemical profile of fraction and compounds: The GC-MS analysis of the pure compounds and semipurified subfractions of *A. cupreata* led to the identification of 12 compounds, of these only dodecanoic acid and tetradecanoic acid have been reported within the genus *Agave*. The chemical constituents with their retention time (RT), molecular weight (MW) and concentration (percentage) are presented in Table 1. The following compounds (Fig. 1) were present in dichloromethane extract from *A. cupreta*: Bis(6-methylheptyl) benzene-1,2-dicarboxylate (Fig. 1a), bis(2-ethylhexyl) benzene-1,2-dicarboxylate (Fig. 1b), 1-(3-ethyloxiran-2-yl) ethenone (Fig. 1c), 2-nitrohexane (Fig. 1d), 5-methyl-2-propan-2-ylcyclohexan-1-ol (Fig. 1e), dodecane (Fig. 1f), dodecanoic acid (Fig. 1g), tetradecanoic acid (Fig. 1h), 2,6-di-tert butyl-4-methylphenol (Fig. 1i), 2-bromooctane (Fig. 1j), methyl 3,5-dicyclohexyl-4-hydroxybenzoate (Fig. 1k) and bis(2-ethylhexyl) decanedioate (Fig. 1l).

Antibacterial activity: The results obtained by the bioautographic technique were shown in Fig. 2. The FdAc5-FdAc26 fractions were active when presenting inhibition halos against *S. aureus* (ATCC 29213). The halos make it possible to determine that the compounds with antibacterial activity present in the FdAc5-FdAc16 fraction are of medium and low polarity, while the active compounds of the FdAc17-FdAc26 fractions are mostly of low polarity.

The FdAc8, FdAc11, FdAc18, FdAc19, FdAc24 and SFdAc7 fractions were evaluated using the broth microdilution technique, to determine their minimum inhibitory concentration (MIC) against sensitive strains and clinical isolates. The results obtained were presented in Table 2.

The FdAc24 fraction was the most active in inhibiting the growth of both sensitive strains and clinical isolates. Likewise, the semi-purified fraction SFdAc7 was effective at concentrations of 0.4 and 0.05 mg/mL against clinical isolates. The FdAc18 fraction showed an MIC of 10 mg/mL against *Staphylococcus* strains, while the FdAc11 fraction only showed

Table 1: Chemical composition by GC-MS of Ac1, Ac2, Ac3 and Ac4 of *A. cupreata*

C/SFS	RT	MW	Compounds	Percentage
Ac1	25.016	390.564	Bis(6-methylheptyl) benzene-1,2-dicarboxylate	100
Ac2	25.003	390.564	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	100
Ac3	6.125	114.14	1-(3-ethyloxiran-2-yl) ethanone	6.448
	6.269	131.17	2-Nitrohexane	6.493
	9.619	156.26	5-Methyl-2-propan-2-ylciclohexan-1-ol	1.046
	9.882	170.33	Dodecane	1.006
	14.907	200.32	Dodecanoic acid	3.983
	17.114	228.37	Tetradecanoic acid	4.875
	24.852	390.564	Bis(6-methylheptyl) benzene-1,2-dicarboxylate	76.149
Ac4	19.721	220.35	2,6-di-terc butyl-4-methylphenol	0.653
	24.241	390.564	Bis(6-methylheptyl) benzene-1,2-dicarboxylate	0.733
	25.443	390.564	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	95.478
	26.408	193.12	2-Bromooctane	0.77
	28.931	426.7	Bis(2-ethylhexyl) decanedioate	0.602
	43.999	316.4	3,5-dicyclohexyl-4-hydroxybenzoate methyl	1.765

FS/C: Semi-purified subfraction/compound, RT: Retention time and MW: Molecular weight

Table 2: Minimum inhibitory concentration (mg/mL) of dichloromethane fractions of *A. cupreata*, against sensitive bacteria and clinical isolates

Strains ATCC	FdAc8	FdAc11	FdAc18	FdAc19	FdAc24	SFdAc7	NC	PC
Staphylococcus aureus	>0.1	32	10	>2	8	>0.4	+	-
Enterococcus faecalis	>0.1	>32	>10	>2	8	>0.4	+	-
Escherichia coli 35218	>0.1	16	>10	>2	8	>0.4	+	-
Escherichia coli 25923	>0.1	>32	>10	>2	8	>0.4	+	-
Enterobacter cloacae	>0.1	>32	>10	>2	8	>0.4	+	-
Salmonella dublin	>0.1	>32	>10	>2	8	>0.4	+	-
Clinical isolates								
Staphylococcus haemolyticus	>0.1	>32	10	>2	8	0.4	+	-
Staphylococcus hominis	>0.1	>32	>10	>2	8	>0.4	+	-
Staphylococcus aureus	>0.1	>32	10	>2	8	0.05	+	-
Escherichia coli	>0.1	>32	>10	>2	8	>0.4	+	-
Klebsiella pneumoniae	>0.1	>32	10	>2	8	>0.4	+	-

NC: Negative control, PC: Positive control: Amikacin (100 µg/mL), +: Bacterial growth and -: No bacterial growth

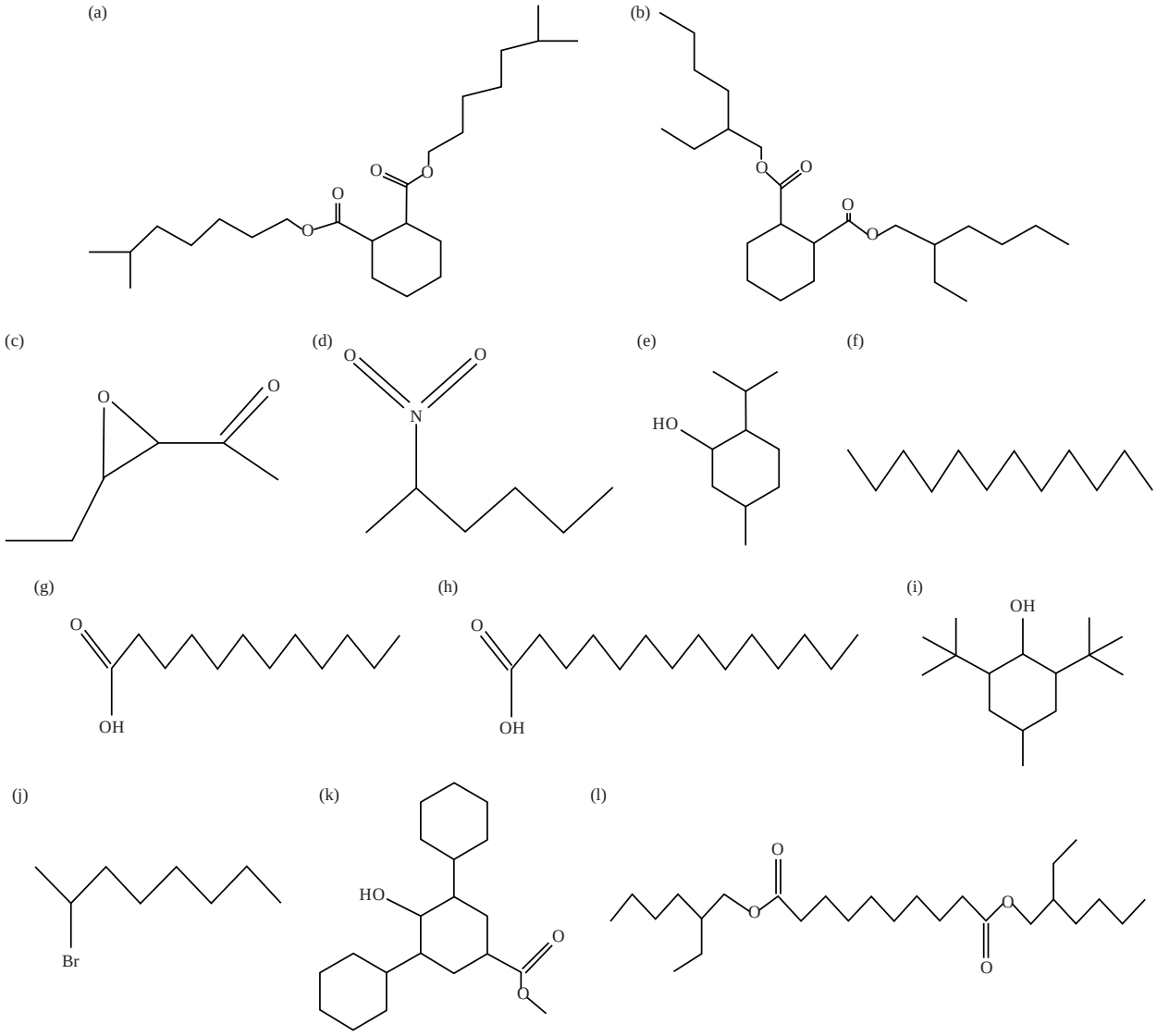

Fig. 1(a-l): Chemical structure of compounds, (a) Bis(6-methylheptyl) benzene-1,2-dicarboxylate, (b) Bis(2-ethylhexyl) benzene-1, 2-dicarboxylate, (c) 1-(3-ethyloxiran-2-yl) ethanone, (d) 2-Nitrohexane, (e) 5-Methyl-2-propan-2-ylcyclohexan-1-ol, (f) Dodecane, (g) Dodecanoic acid, (h) Tetradecanoic acid, (i) 2,6-di-tert butyl-4-methylphenol, (j) 2-Bromooctane, (k) Methyl 3,5-dicyclohexyl-4-hydroxybenzoate and (l) Bis(2-ethylhexyl) decanedioate

antibacterial activity against two ATCC strains. On the other hand, the MIC of the FdAc8 and FdAc19 fractions was higher at the concentrations evaluated.

The semi-purified compounds and fractions (Ac1-Ac4) were subjected to the broth microdilution technique to determine the antibacterial potential after the purification method. Compound Ac2 was able to inhibit Gram-positive strains, while the MIC of Ac1, Ac3 and Ac4 was above the concentrations evaluated. It is important to note that before purification, the FdAc11 fraction, from which the SFdAc7 submeeting and the Ac2 compound came, had MIC greater than 32 mg/mL, this indicates that after purification its MIC decreased 8, 10, 21, 64 and 512 times against *S. aureus* (ATCC), MR *S. haemolyticus* and MR *S. aureus* (Table 3).

Antiulcerative activity: In comparison with normal control (Fig. 3a) and negative control (Fig. 3b), the stomachs of mice treated with FdAc24 (Fig. 3c) and Ac4 (Fig. 3d) presented stomachs with a smaller ulcerated area. Statistical analysis indicated that the FdAc24 fraction and the semipurified Ac4 fraction generated a decrease in the extent of gastric mucosal damage with a protection percentage of 66 and 65%, respectively, compared to the normal control (Table 4).

Table 3: Minimum inhibitory concentration (µg/mL) of the SFdAc7 subfraction and the pure compounds

Strains ATCC	Ac1	Ac2	Ac3	Ac4	NC	PC
Staphylococcus aureus	>300	300	>300	>300	+	-
Enterococcus faecalis	>300	>300	>300	>300	+	-
Escherichia coli 35218	>300	>300	>300	>300	+	-
Escherichia coli 25923	>300	>300	>300	>300	+	-
Enterobacter cloacae	>300	>300	>300	>300	+	-
Salmonella dublin	>300	>300	>300	>300	+	-
Clinical isolates						
Staphylococcus haemolyticus	>300	150	>300	>300	+	-
Staphylococcus hominis	>300	>300	>300	>300	+	-
Staphylococcus aureus	>300	6.25	>300	>300	+	-
Escherichia coli	>300	>300	>300	>300	+	-
Klebsiella pneumoniae	>300	>300	>300	>300	+	-

NC: Negative control, PC: Positive control, Amikacin (100 µg/mL), +: Bacterial growth and -: No bacterial growth

Table 4: Antiulcerogenic activity of *A. cupreata* on the formation of ethanol-induced ulcers in mice

Treatments	Ulceration index (%)	Protection (%)
CN	-	100
NEtOH	56.05±0.29**	-
FdAc24+EtOH	18.90±2.00**	66.27±3.57**
Ac4+EtOH	19.54±2.24**	65.13±4.00**

CN: Normal control treated with vehicle (1% Tween 20), NEtOH: Negative control treated with ethanol (10% by weight), FdAc24+EtOH and Ac4+EtOH: FdAc24 (100 mg/kg) and Ac4 (10 mg/kg), respectively, plus ethanol (10% of body weight), data represent Means±SD, Dunnett's test vs CN control and **p 0.001

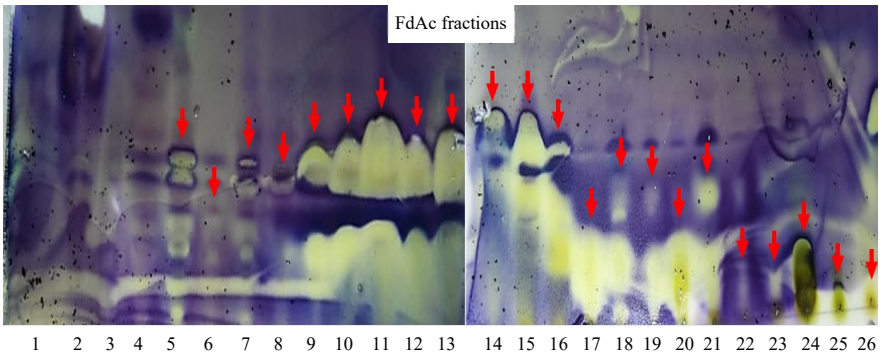

Fig. 2: Bioautography of the FdAc1-FdAc26 fractions of *A. cupreata*
Yellow spots (red arrows) represent bacterial growth inhibition of *S. aureus* and TLC plate eluted with the hexane:acetone system (70:30 v/v)

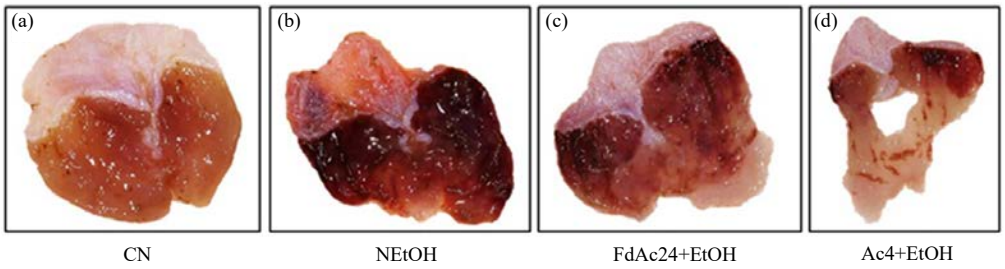

Fig. 3(a-d): Protective effects of *A. cupreata*, were observed with the ethanol-induced gastric ulcer method, (a) Normal control (Tween 20 at 1%), (b) Negative control (ethanol 10% weight), (c) FdAc24 (100 mg/mL) and (d) Ac4 (10 mg/mL)

DISCUSSION

In this study, 12 compounds were characterized from fractions obtained with dichloromethane from *A. cupreata*, only two compounds have been identified in species of the *Agave* genus and 10 have been reported in other plants for medicinal use. In addition, a fraction (FdAb24) presented both antibacterial activity against Gram-positive bacteria and antiulcerogenic activity, which must be characterized to help fight infections caused by antibiotic-resistant bacteria and

gastric ulceration. The identified compounds present in the highest percentage were diisooctyl phthalate (76-100%) and bis(2-ethylhexyl) phthalate (95-100%). Of the identified compounds, only dodecanoic acid and tetradecanoic acid have been reported in *A. decipiens*[15] and *A. sisalana*[16], respectively. The rest of the compounds found in this study have not been isolated within the *Agave* genus, however, they have been found in other plants and organisms such as *Mentha longifolia*[17], *Salvia glutinosa*[18], *Rungia pectinata*[19] and *Areca* palm[20]. The compounds present in the fractions of *A. cupreata* belong to flavonoids and alkaloids, which have many biological properties, including their antibacterial activity. Among the mechanisms of action reported for these compounds, are nucleic acid synthesis and repair inhibition, alteration of the cytoplasmic membrane, inhibition of DNA gyrase, efflux pump inhibition, protein activity inhibition, peptidoglycan biosynthesis inhibition and inhibit bacterial growth by modulating the expression of virulence factors[21,22]. The antibacterial activity results obtained by bioautography indicated that the FdAc5-FdAc26 fractions had antibacterial activity by inhibiting the bacterial growth of *S. aureus*. Likewise, the quantitative antibacterial determination indicated that the FdAc24 fraction of *A. cupreta* had an inhibitory capacity against ATCC bacteria and clinical isolates at a concentration of 8 mg/mL. While the compound Ac2 (bis(2-ethylhexyl) phthalate) at concentrations ranging from 0.0062-0.3 mg/mL inhibited the growth of *Staphylococcus* species (Gram-positive). Gram-negative bacteria have an outer membrane (OM) in their cell wall, which distinguishes them from Gram-positives. The presence of porins in the OM allows the passage of small molecules, but any alteration in the outer membrane, such as changing the hydrophobic properties or mutations in porins and other factors, can create resistance, the reason why Gram-negative bacteria are resistant to a wide range of antibiotic[23].

Gram-positive bacteria have a mesh-like peptidoglycan layer that is more accessible to permeation by plant-derived antibacterials, making them more susceptible to secondary metabolites[24]. There are reports of antibacterial activity of other *Agave* species: Aqueous and methanol extracts of *A. sisalana* had a MIC of 20 mg/mL against *Bacillus atrophaeus, Pseudomonas aeruginosa* and *Enterococcus faecalis*. Similarly, methanol, chloroform, ethyl acetate, n-butanol and n-hexane extracts of *A. attenuata* showed antibacterial activity against *Bacillus subtilis*, *S. aureus* and *Escherichia coli* a MIC of 15.2-252 mg/mL[8]. Likewise, the methanol extract of *A. angustifolia*, presented a MIC of 15 mg/mL against *Streptococcus* and *Shigella dysenteriae*[25].

Unlike other metabolites, the compound Ac2 (bis(2-ethylhexyl) phthalate) does not have bibliographic information that talks about its antibacterial action mechanisms, however, the results obtained are consistent with those reported by Rowshanul Habib and Rezaul Karim[26]. This compound can inhibit Gram-negative and Gram-positive bacteria at concentrations of 128, 64, 32 and 16 µg/mL.

In the same way, flavonoids can protect the human body against ROS-evoked damage and their consumption has potential health. Diosmin, DIO, (diosmetin 7-O-rutinoside), a natural citrus flavone, has displayed remarkable antioxidant, anti-inflammatory and anti-apoptotic activities in human and experimental models[27]. In the present investigation, the antiulcerogenic activity of *A. cupreta* was also evaluated, to know its protective potential. It has been reported that conventional antisecretory drugs can cause adverse effects and relapses; while most herbal medicines reduce offensive effects and are safe, effective, less expensive and globally competitive[28].

In this study, the protective effect of the FdAc24 fraction was evaluated due to its antibacterial efficiency and of the Ac4 subfraction based on its semipurification and quantity obtained. The ethanol-induced gastric ulcer model was used, it allows reproduction of several characteristics of the human condition and therefore provides a means to evaluate agents with possible anti-ulcer actions[27]. The FdAc24 fraction and the semipurified Ac4 subfraction at doses of 100 and 10 mg/mL, respectively, decreased the formation of gastric ulcerations with a protection percentage of 66.27 and 65.13%, respectively.

The present research suggests that the protective effect of the fraction could be due to the presence of flavonoids and alkaloids that it possesses. It has been reported that these metabolites can protect the gastrointestinal mucosa through different mechanisms of action such as decreased production and inhibition of histamine, inhibition of the gastric H^+/K^+ proton pump, increased mucosal blood flow, nitric acid synthesis, increased prostaglandin levels, scavenging of free radicals, inhibition of oxidizing enzymes, reduction of lipid peroxidation, inhibition of gastric acid secretion, inhibition of oxidative damage, mucin synthesis and reduction of levels of Tumor Necrosis Factor Alpha (TNF-α) and Interleukin 6 (IL-6)[29-32]. The antiulcerogenic activity of the semipurified Ac4 subfraction may be due to the presence of bis(2-ethylhexyl) phthalate found in an abundance of 95%; It has been described that this compound has antioxidant activity, thus being able to reduce reactive oxygen species that lead to gastric damage. It can also be perfectly coupled to the active site of lipoxygenase (LOX) causing its inhibition; LOX is known to be a key enzyme in leukotriene biosynthesis, therefore it plays an important role in the pathophysiology of inflammatory diseases[33,34]. Likewise, the presence of butylated

hydroxytoluene (BHT) may be involved. According to Yu *et al.*[35] BHT can decrease the expression of genes related to inflammation such as TNF-α and IL-8, in addition to inhibiting the production of reactive oxygen species. This study reported for the first time the presence of diisooctyl phthalate, bis(2-ethylhexyl) phthalate, 2-nitrohexane, ethanone 1-(3-ethyloxiranyl), myristic acid, lauric acid, 3,5-dicyclohexyl-4-methyl hydroxybenzoate, menthol and dodecane in extracts of *Agave cupreata* leaves from the state of Guerrero, Mexico. The antibacterial activity of agave leaf extract may be mainly due to bis(2-ethylhexyl) phthalate. Further studies are required to elucidate the compounds that may be responsible for the antiulcerogenic activity and the cellular mechanisms involved in this process.

CONCLUSION

The results of this study demonstrated that fractions (FdAc11, FdAc18, FdAc24 and SFdAc7) and compound Ac2 of *Agave cupreata* inhibit the growth of both sensitive strains and clinical isolates. Likewise, it was found that the FdAc24 fraction and the semipurified Ac4 fraction decrease in the extent of gastric mucosal damage with a protection percentage of 66 and 65%, respectively. The chemical profile suggests the presence of compounds (bis(6-methylheptyl) benzene-1,2-dicarboxylate and bis(2-ethylhexyl) benzene-1,2-dicarboxylate) could be responsible for bioactivities assessed in this work.

SIGNIFICANCE STATEMENT

This study contributes to the phytochemical and pharmacological properties of fractions and compounds isolated from dichloromethane extract from *Agave cupreata.* The results indicated that fractions and compounds have antibacterial and antiulcerogenic activities. The fractions and compounds exhibited antibacterial activity against *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli* and *Klebsiella pneumoniae*. The antiulcerogenic effect is evidenced by its significant inhibition of the formation of ulcers in mice models. The results indicated that fractions and compounds supported the ethnopharmacological use of this medicinal plant. Further pharmacological studies will be carried out to provide more accurate elucidation of the mechanism action involved in these activities.

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