International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.1063.1070

Research Article
Antibacterial and Anti-inflammatory Activity of Extracts and Fractions from *Agave cupreata*

[1]Diana Teresa Salazar-Pineda, [1]Natividad Castro-Alarcón, [1]Ma. Elena Moreno-Godínez, [2]María del Pilar Nicasio-Torres, [3]Juanita Pérez-Hernández and [4]Patricia Alvarez-Fitz

[1]Facultad de Ciencias Químico Biólogicas, Universidad Autónoma de Guerrero,
Av. Lázaro Cárdenas S/N Col. La Haciendita CP. 39090 Chilpancingo, Guerrero, México
[2]Centro de Investigación Biomédica del Sur (CIBIS), Instituto Mexicano del Seguro Social (IMSS) Argentina #1,
Col. Centro CP. 62790 Xochitepec, Morelos, México
[3]Estancia Postdoctoral CONACYT-Centro de Desarrollo de Productos Bióticos (CEPROBI), Instituto Politécnico Nacional (IPN),
P.O. Box 24, 62730 Yautepec, Morelos, México
[4]CONACYT-UAGro. Av. Lázaro Cárdenas S/N Col. La Haciendita CP. 39090 Chilpancingo, Guerrero, México

Abstract

Background and Objective: In Mexico, *Agave cupreata* is used in traditional medicine to treatment of inflammatory processes. The aim of this study was to evaluate the antibacterial and anti-inflammatory activities of extracts and fractions obtained from *Agave cupreata* leaves. **Methodology:** The *A. cupreata* leaves were extracted by maceration with solvents of increasing polarity: hexane, dichloromethane and acetone. The fractions of dichloromethane extract were obtained by open column chromatography on silica gel and elution system hexane-acetone 7:3. The antibacterial activity was evaluated using the double dilution agar and microtiter broth dilution methods. The anti-inflammatory activity was tested using TPA-induced mouse ear edema and λ-carrageenan-induced mouse paw edema model. One way ANOVA was used to evaluate the statistical differences. **Results:** The MIC of extracts of hexane and dichloromethane were 16 mg mL^{-1} against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and all clinical isolates tested; for the acetone extract only *Staphylococcus simulans* was susceptible (MIC of 16 mg mL^{-1}). Of the fractions obtained from the dichloromethane extract, the F-34 was the most active against all bacteria tested (MIC of 2-16 mg mL^{-1}). As for the anti-inflammatory activity, the dichloromethane and acetone extracts present an inhibitory effect on the formation of edemas of 64.29% (ED_{50} = 107.55 mg kg^{-1} b.wt.) and 48.82%, respectively when inflammation was induced with carrageenan and being induced by TPA it was 62.47% (ED_{50} = 1.21 mg per ear) and 40.82%. **Conclusion:** The extracts of hexane and dichloromethane from *A. cupreata* leaves exhibited antibacterial activity, for both sensitive strains and clinical isolates methicillin-resistant *Staphylococcus*; in addition, it could be an important source of compounds with anti-inflammatory action.

Key words: *Agave cupreata*, antibacterial, anti-inflammatory, *Agavaceae*, biological activity

Received: April 25, 2017 Accepted: June 10, 2017 Published: October 15, 2017

Citation: Diana Teresa Salazar-Pineda, Natividad Castro-Alarcón, Ma. Elena Moreno-Godínez, María del Pilar Nicasio-Torres, Juanita Pérez-Hernández and Patricia Alvarez-Fitz, 2017. Antibacterial and anti-inflammatory activity of extracts and fractions from *Agave cupreata*. Int. J. Pharmacol., 13: 1063-1070.

Corresponding Author: Patricia Alvarez Fitz, Laboratorio de Toxicología y Salud Ambiental. Facultad de Ciencias Químico Biológica, Universidad Autónoma de Guerrero Chilpancingo, Guerrero, México Tel: 527474725503

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Mexico has the greatest diversity of *Agave* species in the world, with as many as 159 species (119 endemic) which occupy more than 75% of the territory. They are very diverse in the arid and semi-arid provinces of the center and north of the country[1,2]. Agaves are plants of great cultural and economic importance, all their structures are used as food (humans and livestock) and material for the production of fibers but its main use is the manufacture of fermented beverages[2,3]. Currently, the agave metabolites are used for the semi-synthesis of steroidal drugs for the pharmaceutical industry[4]. In addition, to its medicinal use for the treatment of bacterial and inflammatory diseases, (e.g., gastrointestinal and wound infections, urological disorders, dysentery)[5,6], cancer and diabetes[2,5,7]. Scientific studies report that crude extracts of species of Agave such as *A. tequilana*, *A. americana*, *A. angustifolia* and partially purified fractions have anti-inflammatory[8,9], anticancer[7,10,11], antimicrobial, antioxidant[12], antihypertensive[13], immunomodulatory[14], antiparasitary[15,16] and antifungal[17] activities. These activities are due to the secondary metabolites that are present in the extracts and fractions such as fatty acids (ethyl esters), triterpenes, saponins, steroids, tannins, flavonoids, cardiac glycosides and fructans[7,12,18,19].

Among the species of Agave in Mexico is *A. cupreata*, this is an endemic plant naturally distributed in the Balsas Depression, a semiarid region in Guerrero and Michoacán states in Mexico Southwestern[20]. This species is distinguished by copper-coloured spines, broad, light green leaves, strongly serrated and with impressions of spines very marked in the edges[21]. Various parts of the plant are used in traditional medicine; however, its main use is the central head ("piña") which is used to the elaboration of "mezcal", an alcoholic beverage similar to the Mexican tequila[22].

Considering that other species of agave already have corroborated antibacterial and anti-inflammatory effects, it is expected that *A. cupreata* could present similar biological activity, it is important to mention that for this species there are no scientific reports about its biological activity and as regards its phytochemical composition, only its lipid content has been determined[19]. The interest in anti-inflammatory and antibacterial substances of plant origin is increasing because, in some cases, they offer advantages over in some classic drugs, such as the low incidence of side effects[23], for that reason, the objective of this study was to evaluate the antibacterial and anti-inflammatory activity of extracts of *A. cupreata* which will produce knowledge of molecules with biological activity that in the future could potentially be used to obtain new phytodrugs.

MATERIALS AND METHODS

Plant material: The *A. cupreata* leaves were collected in Guerrero State, Mexico in July 2015. The plant material was identified by Dr. Abisaí Josué García Mendoza from Biology Institute of National Autonomous University of Mexico (UNAM), México. A voucher specimen with accession number MEXU-2050 was submitted to the Nation herbarium of Mexico (MEXU) and identified as *Agave cupreata* Trel. and A. Berger.

Plants extracts and fractions: The dried and ground leaves of *A. cupreata* (3.5 kg) were extracted successively by maceration using ascending polarity solvents: Hexane, dichloromethane and acetone (reagent grade, 1.5 L at 24 h, 3 times). The macerated material was filtered, extracts pooled and evaporated under reduced pressure in a rotary evaporator (digital rotary evaporator 410, Puebla, México). The extract yields were 0.069, 0.138 and 0.572%, respectively. The dichloromethane extract was submitted to Open Column Chromatography (OCC) on processing on Kiesegel 60 (Merck, Germany). This procedure was initiated with 100% n-hexane as eluent system and polarity was gradually increased by means of successive additions of acetone to obtain 92 aliquots (150 mL). The fractions obtained were monitored by Thin Layer Chromatography (TLC) (Silica gel 60 F254 Merck, Germany), visualized by Ultraviolet light (UV) to 302 and 365 nm and revealed with acid reagent (Sigma Chemical Co., St. Louis, MO, USA). The aliquots were combined according to characteristics observed by TLC in twenty fractions. Secondary metabolites (antrones, anthraquinones, coumarins, alkaloids, essential oils, pungent compounds, saponins, steroids, lignans and catechins) were determined by qualitative phytochemical analysis of the extracts which were carried out using standard qualitative methods (TLC).

Antibacterial activity

Tested microrganisms: The following six strains were obtained from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 29213, *Enterobacter cloacae* ATCC 700323, *Salmonella dublin* ATCC 9676, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603. Plus nine clinical isolates of Methicillin Resistant *Staphylococcus* (MRS) were obtained from General Hospital of Acapulco, México:

*Staphylococcus haemolitycus (*1129), *Staphylococcus aureus* (1058), *Staphylococcus simulans* (1050), *Staphylococcus haemolitycus* (562), *Staphylococcus hominis* (592), *Staphylococcus hominis* (596), *Staphylococcus haemolitycus* (731), *Staphylococcus haemolitycus* (1036) and *Staphylococcus epidermidis* (1042). All strains were incubated at 37°C in Mueller Hinton agar. The inoculum for 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 CFU mL^{-1}).

Minimal Inhibitory Concentration (MIC): The MIC of extracts and fractions was performed according to that reported by Balouiri *et al.*[24]. A stock solution of extracts and fractions were prepared in dimethyl sulfoxide (DMSO 99.9% of purity at 20%, Sigma-Aldrich grade culture) at a concentration of 2-16 mg mL^{-1}. In the double dilution agar method, the stock solution was mixed with 3 mL of Mueller Hinton agar in Petri plates, in the microtiter broth dilution method the stock solution was mixed with 100 µL of Luria Bertani broth (LB) in microplates (96-wells). Each dilution was inoculated with 2 µL of diluted inoculum. The Petri plate and microplates were incubated at 37°C for 24 h. The microplates were mixed with 200 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Merck, Darmstadt, Germany), the plates were reincubated at 37°C for 15 min after this incubation if the well-turned purple. The MIC was defined as the lowest concentration of the tested substance that inhibited the visible growth of the bacterial strains. Ceftazidime (Glaxo, Bogota, Colombia) was used as standard antibiotics (positive control).

Anti-inflammatory assay

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 seven per cage and were maintained under standard laboratory conditions (25°C, 12 h light/12 h dark and water/food *ad libitum*). The animals were acclimatized to the housing facilities for at 3 weeks before the experiments. All experimental were conducted on a minimum of seven animal and requisites of observation were employed to obtained consistent data.

Carrageenan Footpad Edema (CFE): The procedure was performed according to that reported by Nicasio-Torres *et al.*[25] Acute inflammation was produced by the subplantar injection of 20 µL of λ-carrageenan (1%) in the mouse's right paw and after 60 min were orally (p.o) administered with 150 mg per kg of b.wt., of dichloromethane and acetone extracts or 10 mg per kg of BW of indomethacin (99% purity; Sigma-Aldrich), the control group received water/Tween-20 to 2%. Footpad volume was measured before (time = 0; initial time) and after (1, 3 and 5 h) λ-carrageenan injection using a digital micrometer (Mexico City, Mexico). Footpad edema in the control and treated groups was determined with respect to footpad volume at the time = 0. The percentage of inhibition was calculated according the following expression[26]:

$$\text{Inhibition (\%)} = \frac{\text{Control-treatment}}{\text{Control}}\times 100 \quad (1)$$

TPA-induced mouse ear edema: The effect produced by extracts of *A. cupreata* on the mice ear edema assay was performed according to that reported by Perez-Hernandez *et al.*[27]. Each mouse received 20 µL TPA (12-O-Tetradecanoylphorbol-13-acetate, 2.5 µg per ear), which was topically applied to the right ear leaving the left ear of each mouse as control (20 µL to methanol). After 15 min of TPA application, the treatments (dichloromethane and acetone extracts, 2 mg per ear) and indomethacin (1 mg per ear) were applied (10 µL in the inner ear and 10 µL in the external ear). Five hours after the assay, the animal was sacrificed by cervical dislocation and circular sections of the central portion (6 mm in diameter) were taken from both ears (treated and no-treated), which were weighed to determine the ear edema by differences in weight. The percentage of inhibition was calculated using the Eq. 1[26].

Statistical analysis: The analysis of normal distribution of the data of footpad edema (volume and percentage of inhibition) and mouse ear edema (weight and percentage of inhibition) was analyzed by one way analysis of variance (One Way ANOVA) followed by Tukey test. Values of $p<0.05$ were considered statically significant. Statistical analysis was carried out with a STATA 11.1 program[28].

RESULTS

Antibacterial assay: The antibacterial evaluation of *A. cupeatra* extracts against sensitive strains and clinical isolates of MRS (Table 1) showed that the hexane and dichloromethane extract had antibacterial activity against

Table 1: Minimal inhibitory Concentration (MIC) of extracts and fractions from *Agave cupeatra*

	Extracts			Fractions (mg mL^{-1})		Control (+)		Control (-)
Strains	HE	DE	AE	F-34	F-60	F-84	CAZ	Culture medio
ATCC								
S. aureus	16	16	*	8	8	*	†	+
E. cloacae	*	*	*	*	*	*	†	+
S. dublin	*	*	*	*	*	*	†	+
E. coli	*	*	*	*	16	*	†	+
P. aeruginosa	16	16	*	8	16	16	†	+
K. pneumoniae	*	*	*	*	*	*	†	+
Clinical isolate MRS								
S. haemolitycus 1129	16	16	*	8	16	16	0.5	+
S. aureus 1058	16	16	*	16	16	16	†	+
S. simulans 1050	16	16	16	8	16	16	0.5	+
S. haemolitycus 562	16	16	*	8	16	16	†	+
S. hominis 592	16	16	*	8	16	16	†	+
S. hominis 596	16	16	*	16	16	16	0.5	+
S. haemolitycus 731	16	16	*	8	16	16	†	+
S. haemolitycus 1036	16	16	*	8	16	*	†	+
S. epidermidis 1042	16	16	*	2	16	16	†	+

HE: Hexane extract, DE: Dichloromethane extract, AE: Acetone extract, *S. aureus*: *Staphylococcus aureus* ATCC 29213, *E. cloacae*: *Enterobacter cloacae* ATCC 700323, *S. dublin*: *Salmonella dublin* ATCC 9676, *E. coli*: *Escherichia coli* ATCC 25922, *P. aeruginosa: Pseudomonas aeruginosa* ATCC 27853, *K. pneumoniae*: *Klebsiella pneumoniae* ATCC 700603, CAZ: Ceftazidime, †MIC <0.5 mg mL^{-1}, +Bacterial growth, *MIC>16 mg mL^{-1}

Table 2: Inhibitory activity of dichloromethane and acetone extracts (150 mg kg^{-1} b.wt.) on footpad edema in mice

	Paw edema formation			
Time (h)	Control	Indomethacin	DE	AE
Edema (mm)				
1	0.96±0.07	0.61±0.03	0.66±0.02	0.64±0.03
3	1.16±0.05	0.68±0.01	0.51±0.04*	0.59±0.02
5	1.12±0.08	0.60±0.03	0.40±0.04*	0.55±0.05
Edema inhibition (%)				
1	-	41.07±2.80	31.25±1.68	34.19±3.14
2	-	41.50±1.21	56.22±3.10*	48.82±1.73
3	-	46.30±2.82	64.29±3.88*	47.82±3.09

Mean±Standard Error of Mean (SEM). ANOVA *post hoc* Tukey with n = 7, *p<0.05 when are compared with indomethacin group, DE: Dichloromethane extract, AE: Acetone extract

Staphylococcus aureus, *Pseudomonas aeruginosa* and all clinical isolates MRS at a concentration of 16 mg mL^{-1}. The extract of acetone displayed low activity against all strains examined. The fraction F-34 inhibited the growth of all clinical isolates MRS, being more active against *Staphylococcus epidermidis* 1042 with a MIC of 2 mg mL^{-1} (Table 1).

Anti-inflammatory assay: The dichloromethane and acetone extracts were analyzed in the CFE and TPA-induced mouse ear edema methods. In the first model was observed that after theadministration of the proinflammatory, the maximum effect in the formation of edema was at 3 and 5 h.

The dichloromethane extract significantly inhibited (p<0.05) the formation of subplantar edema in 56.22 and 64.29% at 3 and 5 h, respectively (Table 2) with a level of edema of 0.51 and 0.40. This extract showed a dose-dependent effect with a median effective dose (ED_{50}) of 107.55 mg per kg of b.wt., (Fig. 1). Statistically the acetone extract presented an anti-inflammatory effect similar to indomethacin, the highest anti-inflammatory activity was observed at 3 h.

Table 3: Inhibitory activity of dichloromethane and acetone extracts on TPA-induced mouse ear edema

Treatments	Doses (mg per ear)	Edema (mg)	Inhibition (%)
Control	-	12.17±0.69	-
Indomethacin	1.00	8.31±0.54	37.21±3.76
DE	2.00	4.57±0.54*	62.47±4.48*
AE	2.00	7.20±0.48	40.82±3.96

Mean±Standard Error of Mean (SEM) (n = 7). ANOVA *post hoc* Tukey with n = 7, *p<0.05 when compared with indomethacin group, DE: Dichloromethane extract, AE: Acetone extract

In the TPA-induced mouse ear edema method, the dichloromethane extract at a dose of 2 mg per ear inhibited the formation of edema in 62.47% similar to that obtained in CFE; the acetone extract at this same dose inhibits in 40.82% similar to indomethacin (Table 3). The dichloromethane extract showed a dose-dependent effect with an ED_{50} of 1.21 mg per ear (Fig. 2).

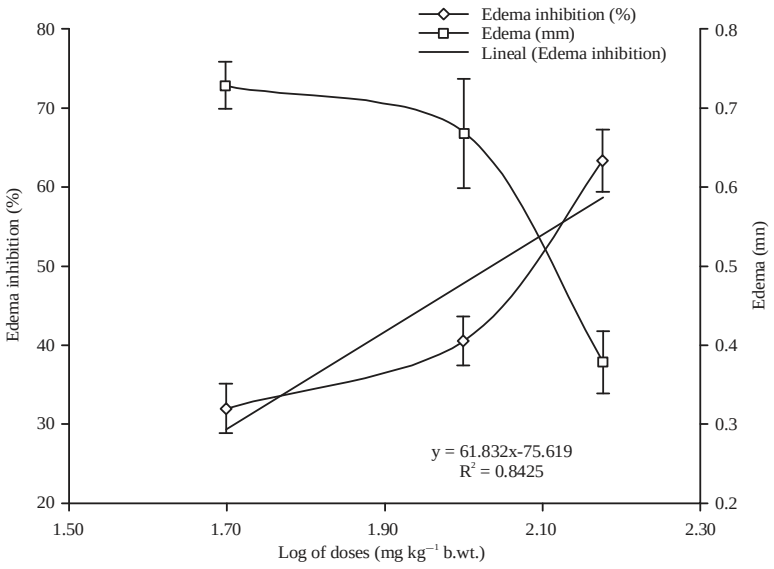

Fig. 1: Effects of the dichloromethane extract of *A. cupreata*, on CFE

The "best-fit" line shown was generated by linear regression of the data (n = 7), squares of correlation coefficient (R^2) regression equations are reported. Vertical bars represent the standard error of the means (SEM)

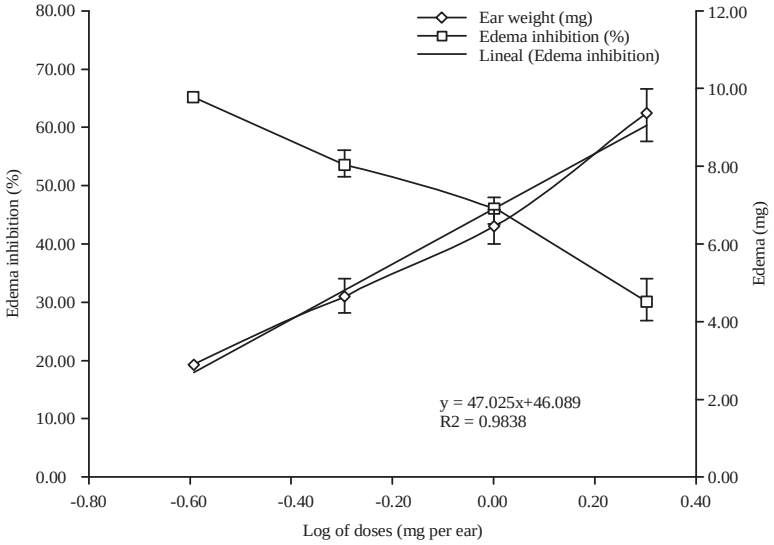

Fig. 2: Effects of the dichloromethane extract of *A. cupreata*, on auricular edema

The "best-fit" line shown was generated by linear regression of the data (n = 7), squares of correlation coefficient (R^2) regression equations are reported. Vertical bars represent the standard error of the means (SEM)

DISCUSSION

Historically, it has been recorded that agave products possess biological activity. In this study, antibacterial and anti-inflammatory action was demonstrated in hexane, dichloromethane and acetone extracts from *A. cupreata*.

In terms of antibacterial activity was found that hexane and dichloromethane extracts have a MIC 8-16 mg mL^{-1} against Gram positive and Gram negative species. It is important to mention that there are no reports in which the antibacterial activity of *A. cupreata* is evaluated, so it is not possible to make comparisons for this particular species. However, study has been done on polar extracts and similar MICs were observed in this study, for example, Ahumada-Santos *et al.*[12] determined that the methanolic extract of *A. impressa*, *A. ornithobroma*, *A. rzedowskiana*,

A. tequilana, *A. schidigera* and *A. angustifolia* had MICs 5-15 mg mL^{-1} against ATCC strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and clinical isolates (*Streptococcus* group A-4, *Salmonella enterica* Typhi and *Shigella dysenteriae*). On the other hand, Hammuel *et al.*[18] determined the MIC of polar extracts (aqueous and methanolic) of *A. sisalana* and found activity against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Streptococcus pyogenes* and *Candida albicans* to be in the range of 10-20 mg mL^{-1}.

When the dichloromethane extract was fractionated, it was observed that the MIC of the fractions was lower in comparison with the total extract. Especially in F-34 which was the fraction with the best activity with MIC 2-16 mg mL^{-1}, being up to eight times more active against *Staphylococcus epidermidis* 1042 (methicillin-resistant clinical isolates).

The wide diversity and large number of molecules that may be contained in a whole extract raises the possibility that the constituent molecules may react to generate effects such as synergism or antagonism. Thus, some individual molecules may have stronger effects than the whole extract (antagonist within the extract) or a reduced effect (is a synergistic factor in the extract)[29].

According to the preliminary phytochemical profile performed in this study, the presence of antrones, anthraquinones, coumarins, alkaloids, essential oils, pungent compounds and saponins were detected. It is probable that the antibacterial action observed in the extracts is caused by these metabolites. For example, the alkaloids are capable of intercalate the DNA, resulting in the alteration of the division and cell death, it can also reduce the motility of *Escherichia coli* (UPEC) and has been shown to increase the antibiotic's capacity to destroy the bacterial biofilm[30,31], triterpenoids inhibit efflux pumps in *Enterococcus faecalis* and methicillin resistant *Staphylococcus aureus*[32].

On the other hand, it is known that bacterial infections can trigger inflammatory processes as a result of some structural components, among which are the peptidoglycans, lipoteicoic acid and lipopolysaccharides[33,34]. In addition to other components such as *Staphylococcus aureus* leucotoxins enhance the host inflammatory response and influence the outcome of infection [35].

Results obtained by other studies using different methodologies showed that the *Agave* genus is characterized by good anti-inflammatory activity[9,36,37]. On CFE, the methanolic extract of *A. cantala* (Roxb.) and *A. intermixta* Trel. leaf and capable of generating between 46.15 and 81.4% inhibition at concentrations of 200-500 mg per kg of b.wt.[36,37], on the other hand, in the TPA-induced mouse ear edema method the extracts of acetone and methanol from *A. tequilana*, *A. americana*, *A. angustifolia* and *A. cantala* generate 51-81% inhibition between 3-6 mg per ear[9,37].

In this study, it was observed that dichloromethane and acetone extracts of *A. cupreata* leaves were able to inhibit inflammation with similar effect. It will be interesting to evaluate the anti-inflammatory activity of fractions and/or pure compounds, this behavior would be similar to those reported in other studies, in which compounds such as saponins and phytosterols were elucidated[8,9], so it is probable that the saponins identified in the acetone extract in this study are responsible for exercising the action anti-inflammatory; whereas in dichloromethane extract, the presence of alkaloids may be those that exert the action, because according to Beg *et al.*[38], the alkaloids present in other plant species exert this biological activity.

It is known that the inflammatory response promoted by carrageenan is biphasic type, the early phase in edema formation occurs within the first characterized by the release of histamine and serotonin; the second phase is associated with the release of prostaglandins, bradykinin and protease with a maximum effect occurring about 3 h after carrageenan injection[23,38]. In this study, the dichloromethane extract and acetone extract that are present possess a potent anti-inflammatory activity, both in the first and second phase.

In the case of TPA, it is able to activate protein kinase C, which activates other enzymatic cascades in turn, leading to release of different molecules. This stimulates vascular permeability, vasodilation, polymorphonuclear leukocytes migration, release of histamine and serotonin as well as moderate synthesis of inflammatory eicosanoids by cyclooxygenase and 5-lipoxygenase enzymes[23,38]; therefore, it is likely that the good anti-inflammatory activity of the *Agave* extracts is due to the inactivation of these enzymes or their products.

CONCLUSION

In this study, the antibacterial and anti-inflammatory activities of hexane, dichloromethane and acetone extract of *A. cupreata* was reported for the first time. These activities are in agreement with the ethnomedical use. Futher studies on *A. cupeatra* could contribute to discover new antibacterial and anti-inflammatory agents. To continue this study, must

isolate compounds from dichloromethane extract and evaluate the antibacterial and anti-inflammatory activity of the pure compounds.

SIGNIFICANCE STATEMENTS

This study reports the antibacterial and anti-inflammatory activities of extracts and fractions from *Agave cupreata*. The present contribution provides quantitative data relating with biological activity from *Agave cupreata*. This study will help in the future in the design of new phytodrugs.

ACKNOWLEDGMENTS

This study received financial support provided by grant Fondos Mixtos CONACyT-Gobierno del Estado de Guerrero (No. 249671). Diana Teresa Salazar Pineda thanks Beca CONACyT (No. 402686). The authors would like to thank Margarita Aviles and Macrina Fuentes of the ethnobotanical garden of INAH, at Cuernavaca, Morelos.

REFERENCES

1. Garcia, A.J., 2007. Los agaves de Mexico. Ciencias, 87: 14-23.
2. Garcia-Mendoza, A.J., 2011. Flora del valle de Tehuacan-Cuicatlan: Agavaceae. Universidad Nacional Autonoma de Mexico, Mexico, DF., ISBN: 9786070225666, Pages: 95. Mexico, DF., ISBN: 9786070225666, Pages: 95.
3. Delgado-Lemus, A., A. Casas and O. Tellez, 2014. Distribution, abundance and traditional management of *Agave potatorum* in the Tehuacan Valley, Mexico: Bases for sustainable use of non-timber forest products. J. Ethnobiol. Ethnomed., Vol. 10. 10.1186/1746-4269-10-63.
4. Sparg, S.G., M.E. Light and J. van Staden, 2004. Biological activities and distribution of plant saponins. J. Ethnopharmacol., 94: 219-243.
5. Torres, I., J. Blancas, A. Leon and A. Casas, 2015. TEK, local perceptions of risk and diversity of management practices of *Agave inaequidens* in Michoacan, Mexico. J. Ethnobiol. Ethnomed., Vol. 11. 10.1186/s13002-015-0043-1.
6. Cornara, L., A. La Rocca, S. Marsili and M.G. Mariotti, 2009. Traditional uses of plants in the Eastern Riviera (Liguria, Italy). J. Ethnopharmacol., 125: 16-30.
7. Santos-Zea, L., A. Maria Leal-Diaz, E. Cortes-Ceballos and J.A. Gutierrez-Uribe, 2012. Agave (*Agave* spp.) and its traditional products as a source of bioactive compounds. Curr. Bioactive Compounds, 8: 218-231.
8. Hernandez-Valle, E., M. Herrera-Ruiz, G.R. Salgado, A. Zamilpa and M.L. Ocampo *et al*., 2014. Anti-inflammatory effect of 3-O-[(6'-O-palmitoyl)-β-D-glucopyranosyl sitosterol] from *Agave angustifolia* on ear edema in mice. Molecules, 19: 15624-15637.
9. Monterrosas-Brisson, N., M.L. Ocampo, E. Jimenez-Ferrer, A.R. Jimenez-Aparicio and A. Zamilpa *et al*., 2013. Anti-inflammatory activity of different agave plants and the compound cantalasaponin-1. Molecules, 18: 8136-8146.
10. Casillas, F.R., A.O. Cardenas, C.R. Morales, M.J.V. Star and D.E. Cruz-Vega, 2012. Cytotoxic activity of *Agave lechuguilla* Torr. Afr. J. Biotechnol., 11: 12229-12231.
11. Khade, K.V., H. Dubey, C.R. Tenpe, P.G. Yeole and A.M. Patole, 2011. Anticancer activity of the ethanolic extracts of *Agave americana* leaves. Pharmacologyonline, 2: 53-68.
12. Ahumada-Santos, Y.P., J. Montes-Avila, M. de Jesus U ribe-Beltran, S.P. Diaz-Camacho and G. Lopez-Angulo *et al*., 2013. Chemical characterization, antioxidant and antibacterial activities of six *Agave* species from Sinaloa, Mexico. Ind. Crops Prod., 49: 143-149.
13. Duncan, A.C., A.K. Jager and J. van Staden, 1999. Screening of Zulu medicinal plants for angiotensin converting enzyme (ACE) inhibitors. J. Ethnopharmacol., 68: 63-70.
14. Chen, P.Y., Y.C. Kuo, C.H. Chen, Y.H. Kuo and C.K. Lee, 2009. Isolation and immunomodulatory effect of homoisoflavones and flavones from *Agave sisalana* Perrine ex Engelm. Molecules, 14: 1789-1795.
15. Thakur, C.P., S. Narayan, S. Bahadur, M. Thakur and S.N. Pandey *et al*., 2015. Anti-leishmanial activity of *Agave americana* L.-A traditional Indian medicinal plant. Indian J. Tradit Knowle., 14: 658-663.
16. Guerra, J.O., A. Meneses, A.M. Simonet, F.A. Macias, C. Nogueiras, A. Gomez and J.A. Escario, 2008. Saponinas esteroidales de la planta *Agave brittoniana* (*Agavaceae*) con actividad contra el parasito *Trichomona vaginalis*. Rev. Biol. Trop., 56: 1645-1652.
17. Verastegui, A., J. Verde, S. Garcia, N. Heredia, A. Oranday and C. Rivas, 2008. Species of *Agave* with antimicrobial activity against selected pathogenic bacteria and fungi. World J. Microbiol. Biotechnol., 24: 1249-1252.
18. Hammuel, C., G.G. Yebpella, G.A. Shallangwa, A.M. Magomya and A.S. Agbaji, 2011. Phytochemical and antimicrobial screening of methanol and aqueous extracts of *Agave sisalana*. Acta Pol. Pharm., 68: 535-539.
19. Martinez-Aguilar, J.F. and A. Pena-Alvarez, 2009. Characterization of five typical agave plants used to produce mezcal through their simple lipid composition analysis by gas chromatography. J. Agric. Food Chem., 57: 1933-1939.
20. Gentry, H.S., 1982. Agaves of Continental North America. University of Arizona Press, Arizona, USA., ISBN-13: 9780816507757, Pages: 670.

21. Avendano-Arrazate, C.H., L. Iracheta-Donjuan, J.C. Godinez-Aguilar, P. Lopez-Gomez and A. Barrios-Ayala, 2015. [Morphological characterization of endemic *Agave cupreata* species of Mexico]. Phyton-Int. J. Exp. Bot., 84: 148-162.
22. Aguirre-Dugua, X. and L.E. Eguiarte, 2013. Genetic diversity, conservation and sustainable use of wild *Agave cupreata* and *Agave potatorum* extracted for mezcal production in Mexico. J. Arid Environ., 90: 36-44.
23. Gomez Estrada, H.A., K.M. Gonzalez Ruiz and J.D. Medina, 2011. [Anti-inflammatory activity of natural products]. Bol. Latinoam Caribe Plant. Med. Aromat., 10: 182-217.
24. Balouiri, M., M. Sadiki and S.K. Ibnsouda, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharma. Anal., 6: 71-79.
25. Nicasio-Torres, M.D.P., J. Perez-Hernandez, M. Gonzalez-Cortazar, M. Meckes-Fischer, J. Tortoriello and F. Cruz-Sosa, 2016. Production of potential anti-inflammatory compounds in cell suspension cultures of *Sphaeralcea angustifolia* (Cav.) G. Don. Acta Physiol. Planta., 38: 209-209.
26. Nile, S.H. and S. Won Park, 2013. Optimized methods for *in vitro* and *in vivo* anti-inflammatory assays and its applications in herbal and synthetic drug analysis. Mini Rev. Med. Chem., 13: 95-100.
27. Perez-Hernandez, J., M. Gonzalez-Cortazar, S. Marquina, M. Herrera-Ruiz and M. Meckes-Fischer *et al*., 2014. Sphaeralcic acid and tomentin, anti-inflammatory compounds produced in cell suspension cultures of *Sphaeralcea angustifolia*. Planta Medica, 80: 209-214.
28. Stata Corp, 2010. Stata statistical software version 11.1. StataCorp LLC., College Station TX.
29. Wagner, H. and G. Ulrich-Merzenich, 2009. Synergy research: Approaching a new generation of phytopharmaceuticals. Phytomedicine, 16: 97-110.
30. Dusane, D.H., Z. Hosseinidoust, B. Asadishad and N. Tufenkji, 2014. Alkaloids modulate motility, biofilm formation and antibiotic susceptibility of uropathogenic *Escherichia coli*. PloS One, Vol. 9. 10.1371/journal.pone.0112093.
31. Upadhyay, A., I. Upadhyaya, A. Kollanoor-Johny and K. Venkitanarayanan, 2014. Combating pathogenic microorganisms using plant-derived antimicrobials: A mini-review on the mechanistic basis. Biomed. Res. Int., 10.1155/2014/761741.
32. Ramalhete, C., G. Spengler, A. Martins, M. Martins and M. Viveiros *et al*., 2011. Inhibition of efflux pumps in meticillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* resistant strains by triterpenoids from *Momordica balsamina*. Int. J. Antimicrob. Agents, 37: 70-74.
33. Plociennikowska, A., A. Hromada-Judycka, K. Borzecka and K. Kwiatkowska, 2015. Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling. Cell. Mol. Life Sci., 72: 557-581.
34. Gutierrez-Venegas, G. and P. Cardoso-Jimenez, 2006. Acido lipoteicoico: Receptores y mecanismo de transduccion. REB., 25: 41-49.
35. Malachowa, N., S.D. Kobayashi, K.R. Braughton, A.R. Whitney, M.J. Parnell, D.J. Gardner and F.R. DeLeo, 2012. *Staphylococcus aureus* leukotoxin GH promotes inflammation. J. Infect. Dis., 206: 1185-1193.
36. Reddy, G.K., S.M. Lakshmi, C.K.A. Kumar, D.S. Kumar and T.L. Srinivas, 2013. Evaluation of anti-inflammatory and antioxidant activity of methanolic extract of *Agave cantala* roxb. J. Global Trends Pharm. Sci., 4: 1300-1309.
37. Garcıa, M.D., A.M. Quılez, M.T. Saenz, M.E. Martınez-Domınguez and R. de La Puerta, 2000. Anti-inflammatory activity of *Agave intermixta* Trel. and *Cissussicyoides* L., species used in the Caribbean traditional medicine. J. Ethnopharmacol., 71: 395-400.
38. Beg, S., S. Swain, H. Hasan, M.A. Barkat and M.S. Hussain, 2011. Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. Pharmacogn. Rev., 5: 120-137.