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In vitro anthelmintic and antibacterial effect of *Kalanchoe daigremontiana* leaves and stems hydroalcoholic extract

Efecto antihelmíntico y antibacteriano *in vitro* del extracto hidroalcohólico de hojas y tallos de *Kalanchoe daigremontiana*

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ABSTRACT

Five concentrations (200 to 12.5 mg mL $^{-1}$) of *Kalanchoe daigremontiana* hydroalcoholic extract, were used to determine its anthelmintic and antibacterial effect *in vitro*. To determine the anthelmintic effect, eggs hatching inhibition, inhibition of motility and larval mortality tests were performed *Haemonchus contortus* (HC). The determination of the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration allowed to evaluate the antibacterial activity of extract on *L. monocytogenes, S. typhi, P. aeruginosa, S. choleraesuis, B. subtilis, E. coli and S. aureus*. *Kalanchoe daigremontiana* hydroalcoholic extract inhibited the 99.5% of HC eggs hatching and reduced the motility of 85.2% of L3 larvae at 400 mg mL $^{-1}$. The lethal concentrations 50 and 90 for the inhibition of hatching were 66.5 and 87.3 mg mL $^{-1}$ and 1.5 and 240.9 mg mL $^{-1}$, for the inhibition of motility. The extract showed activity on Gram positive and Gram-negative bacteria, determining a MIC of 100 mg mL $^{-1}$ on *P. aeruginosa* and *L. monocytogenes* and 0.781 mg mL $^{-1}$ for *B. subtilis* and *S. aureus*. These results indicate that the hydroalcoholic extract of *Kalanchoe daigremontiana* has a potential anthelmintic and antibacterial effect and that it could be used as a biological control strategy.
Keywords: *Kalanchoe daigremontiana*, hydroalcoholic extract, anthelmintic, antibacterial.

RESUMEN

Cinco concentraciones (200 a 12.5 mg mL $^{-1}$) del extracto hidroalcohólico de *Kalanchoe daigremontiana* fueron usadas para determinar su efecto antihelmíntico y antibacteriano *in vitro*. Para determinar el efecto antihelmíntico se realizaron las pruebas de inhibición de la eclosión, inhibición de la motilidad y mortalidad larvaria sobre *Haemonchus contortus* (HC). La determinación de la Concentración Mínima Inhibitoria y Concentración Mínima Bactericida, permitieron evaluar la actividad antibacteriana del extracto sobre *L. monocytogenes, S. typhi, P. aeruginosa, S. choleraesuis, B. subtilis, E. coli* and *S. aureus.* El extracto hidroalcohólico de *Kalanchoe daigremontiana* a 400 mg mL $^{-1}$ inhibió la eclosión de huevos de HC en un 99.5%, y redujo la motilidad del 85.2% de larvas L3 del mismo nematodo. Las concentraciones letales 50 y 90 para la inhibición de la eclosión fueron de 66.5 y 87.3 mg mL $^{-1}$ y de 1.5 y 240.9 mg mL $^{-1}$,

para la inhibición de la motilidad. El extracto mostró actividad sobre bacterias Gram positivas y Gram negativas, determinándose una MIC de 100 mg mL $^{-1}$ sobre *P. aeruginosa* y *L. monocytogenes* y 0.781 mg mL $^{-1}$ para *B. subtilis* and *S. aureus.* Estos resultados indican que el extracto hidroalcohólico de *Kalanchoe daigremontiana* presenta potencial efecto antihelmíntico y antibacteriano y que podría ser utilizado como una estrategia de control biológico.
Palabras clave: *Kalanchoe daigremontiana*, extracto hidroalcohólico, antihelmíntico, antibacteriano.

INTRODUCTION

Central Mexico is a well-known area of sheep breeding, mainly for the meat market. Meat yield and quality, and therefore the economic viability of the industry, depends on the health of the animals. Sheep production requires a continuous vigilance for infection by parasitic nematodes, since their presence impacts on meat productivity Alcala *et al*., (2016). Abomasal worms, especially *Haemonchus contortus* [barber's pole worm; Nematoda: Strongylida] (Aguilar *et al.,* 2016; Castillo *et al*., 2017) are the main nematodes causing gastrointestinal disease to sheep in Mexico*.* Several anthelmintic products have been used to address this problem, many of which have been reported to cause resistance (González *et al*., 2017). On the other hand, traditional medicine in veterinary, especially the use of plant extracts, has been proposed as an environmental-friendly alternative against this parasitic infection (Hernández *et al*., 2011; López *et al*., 2008; Abdelfatta *et al*., 2017).

The use of plants as anthelmintic agents is well documented. Among the species used for this purpose are *Chenopodium album* (Bashir *et al*., 2017*)*, *Digitaria insularis* (Santos *et al*., 2017), *Artemisa parviflora (*Irum-S *et al*., 2017), *Ziziphus jujube (*Preet *et al., 2017), Acacia cochliacantha Argemone mexicana, Taraxacum officinale, Ruta chalepensis,* and *Tagetes filifolia* (Olmedo *et al*., 2017). *Kalanchoe daigremontiana* (also known as "mother of thousands") is an endemic plant in Mexico (Kolodziejczyk *et al*., 2017) with several reported uses in human herbal medicine (Hamburger *et al*., 2017) and various active compounds have been found in the extracts of the leaves and stems of this plant in particular (Huang *et al*., 2013). There are reports on the use of *K. daigremontiana* as an antimicrobial agent. Some of the genera *Kalanchoe* isolated compounds and functions follow (Table 1). Therefore, this study was aimed to evaluate the anthelminthic and antibacterial capacity of a hydroalcoholic extract from *K. daigremontiana.*

MATERIALS AND METHODS

Plant material collection and species identification*: K. daigremontiana* specimens were collected in Ulapa de Melchor Ocampo, Tetepango, Hidalgo, in Central Mexico (latitude 20.142500, longitude −99.167778). Local inhabitants have traditionally used this plant as an herbal medication. Whole plants (roots, leaves, and stems) were collected phenological stage of flowering and prepared for transportation. Species identification was

performed in the botany laboratory of the Institute of Biological Sciences of the Universidad Autónoma del Estado de Hidalgo. A representative voucher specimen of the plant material was deposited in the herbarium of the Institute of Biological Sciences of the Universidad Autónoma del Estado de Hidalgo, and the identification code 06 was assigned to it.

Table 1. Insolated compounds and functions of some *Kalanchoe* genera plants

Species	Isolated compounds	Function	Reference
Kalanchoe pinnata	KPB-100 and KPB-200	inhibitors of HHV-2 and VACV	Cryer *et al*., 2017
Kalanchoe pinnata	Bryophyllin C	Insecticidal	Supratman *et al*., 2000
Kalanchoe daigremontiana×tubiflora	Methyl daigremonate	Insecticidal	Supratman *et al*., 2001
Kalanchoe daigremontiana × *butiflora*	Bryophyllin A	Anti-tumor promoting activity	Supratman *et al*., 2001
Kalanchoe prolifera	Kaempferol, Quercetin, Quercetin-3-O-β-D-glucopyranoside, Kaempferol-3-O-β-D-glucopyranoside, Kaempferol-3-O-α-L-rhamnoside , Quercetin-3-O-sophoriside, Quercetin-3-O-rutinoside	Citotoxic activity against leukimia cells	Aisyah *et al*., 2017
Kalanchoe pinnata	Flavonoids in methanol extract	In vitro anti-diabetic activity	George *et al*., 2018
Kalanchoe daigremontiana	11α,19-dihydroxytelocinobufagin, bersaldegenin-1-acetate, bersaldegenin-1, 3,5-orthoacetate, 19-acetyloxy-11α-hydroxy-12-oxo-telocinobufagin, 19-acetyloxy-1β-hydroxytelocinobufagin	Antioxidant	Kolodziejczyk-Czepas *et al*., 2016

Kalanchoe daigremontiana	Bufadienolides such as 11α,19-dihydroksytelocinobufagin, bersaldegenin-1-acetate, daigredorigenin-3-acetate,	Inhibition of amidolytic activity of thrombin	Kolodziejczyk-Czepas *et al*., 2017
Kalanchoe daigremontiana	Bersaldegenin-1,3,5-orthoacetate	increasing apopthosis in deteriorated cells and promoting celular death	Stefanowicz-Hajduk *et al*., 2020
Kalanchoe pinnata	Quercitrin	Antileishmanial activity	Muzitano *et al*., 2006
Kalanchoe thrysiflora, Kalanchoe marmorata	3-oxo-olean-12-ene, β-sitosterol	Selective cytotoxic activity on MCF7 cells	Singab *et al*., 2012
Kalanchoe tubiflora	kalanchosine dimalate	Citotoxic activity	Huang *et al*., 2013
Bryophyllum pinnatum	Bersaldegenin-1,3,5-orthoacetate	In Vitro enzymes inhibition activity, for modern pharmaceuticals	Prasad Pandey *et al*., 2020

Extract preparation: One-hundred grams of dried leaves and steams (4-6 mm) were macerated with 3000 mL of extraction solvent (30% methanol, 70% water); after 72 h, the extract was separated from the solid residue using a filter paper (Whatman™ qualitative filter paper, grade 1), and the solvent was removed by distillation under reduced pressure in a BÜCHI™ R-210 (Flawil, Germany) rotary evaporator, following the methodology described by Rivero-Pérez *et al*., (2016). The concentrations evaluated for egg hatching and larval motility inhibition, as well as larval mortality, were 200, 100, 50, 25, and 12.5 mg mL^{-1}. Dried extracts (35 g) were stored at −20 °C until used.

Anthelmintic activity

H. contortus (HC) eggs and L3-stage larvae were used to evaluate anthelmintic activity. Parasites of the strain INIFAP were obtained from the National Institute for Forestry, Animal and Agronomic Research (INIFAP).

Egg hatching test

HC eggs were obtained following a protocol approved by the Bioethics Committee of the Institute of Agricultural Sciences of the Universidad Autonoma del Estado de Hidalgo. Briefly, a Hampshire lamb (3-months and 37 kg PV), clinically healthy and free of gastrointestinal nematodes, was infested with HC L3-stage larvae (350 larvae kg^{-1} PV). Twenty-one days after infestation, fecal samples were collected and the number of eggs per gram of feces was determined by the McMaster method (Cordero-Miguel *et al*., 2000). To recover HC eggs, the methodology described by Olmedo *et al*., (2017) was followed. Thirty grams of feces were washed with distilled water in 200-, 100-, 75-, and 37-µm sieves and concentrated in the 37-µm sieve. The material retained in the last sieve was washed with 6 mL of saturated saline solution and centrifuged at 3000 rpm for 3 min. The supernatant was discarded, and the sediment was washed three times with distilled water to obtain free eggs.

A 96-well ELISA plate was used for the assay. Each well was added with 150-200 eggs in 50 µL of distilled water and 50 µL of extract (200, 100, 50, 25, or 12.5 mg mL^{-1}). Each extract concentration was assayed with four replicates, using Ivermectin (5 mg mL^{-1}) and distilled water as positive and negative controls, respectively. The plates were incubated at 30 °C for 48 h in a constant-humidity chamber. After incubation, ten 10-µL aliquots were observed under the microscope to count the number of unhatched eggs and larvae (dead or alive) per well. Finally, the percentage of egg hatching inhibition was calculated using the equation 1.

$$\frac{\text{Number of eggs in the well}}{\text{Number of L1 larve + Number of eggs in the well}} \times 100 \quad (1)$$

Larval mortality test

Feces from infested lambs were mixed with distilled water and polyurethane foam (1.5 × 1.5 × 0.5 cm) and incubated for 10 days at room temperature (15-20 °C). After incubation, HC L3-stage larvae were recovered following the Baermann technique. L3-stage larvae were unsheathed with 3% sodium hypochlorite (NaClO) for one minute and washed three times with distilled water to remove residual NaClO.

The assay was performed in 96-well plates. Each well was added with 150-200 larvae suspended in 50 µL of water and 50 µL of *K. daigremontiana* extract (200, 100, 50, 25, or 12.5 mg mL^{-1}). Each extract concentration was assayed with four replicates, using Ivermectin (5 mg mL^{-1}) and distilled water as positive and negative controls, respectively.

The plates were incubated in a constant-humidity chamber at 30° C for 72 h. After incubation, ten 10-µL aliquots were observed under the microscope to determine the number of larvae alive or dead per well. Mortality rate for each extract concentration was determined using equation 2:

$$\frac{\text{Number of dead larvae in the well}}{\text{Total of larvae in the well}} \times 100 \qquad (2)$$

Larval motility test

For this assay, unsheathed L3-stage HC larvae were placed in 96-well plates. Each well was added with 150-200 larvae suspended in 50 µL of water and 50 µL of extract (200, 100, 50, 25, or 12.5 mg mL^{-1}). Each extract concentration was assayed with four replicates, using Ivermectin (5 mg mL^{-1}) and distilled water as positive and negative controls, respectively. The plates were incubated in a constant-humidity chamber at 30° C for 72 h. After incubation, 10-µL aliquots were observed under the microscope to determine the number of alive/dead, motile/immobile larvae per well. The rate of motility inhibition for each extract concentration was determined using equation 3:

$$\frac{\text{Number of live larvae in each well} - \text{Larvae with movement in each well}}{\text{Total number of larvae in each well (live and dead)}} \times 100 \qquad (3)$$

Antibacterial activity

The microorganisms used to determine the antibacterial activity of the hydroalcoholic extract from *K. daigremontiana* were *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 9027), *Listeria monocytogenes* (ATCC 19113), and *Escherichia coli* (ATCC 35218).

The broth microdilution method described by Kaewpiboo *et al*., (2012) was used with some modifications to determine the minimal inhibitory concentration (MIC) of the *K. daigremontiana* hydroalcoholic extract. Two-fold serial dilutions of *K. daigremontiana* hydroalcoholic extract (from 400 to 0.781 mg mL^{-1}) were prepared by duplicate (100 µL per well). A bacterial cell suspension was adjusted to 0.5 McFarland units (approximately 1.5×10^6 colony forming units [CFU] mL^{-1}). A 10-µL aliquot was added to each well. Kanamycin (AppliChem 4K10421™) was used as a positive control (64-0.5 µg mL^{-1}) and nutritive broth was used as a negative control. Plates were incubated at 37 °C under agitation (70 rpm) for 24 h.

After incubation, 20 µL of 0.04% (w/v) p-iodonitrotetrazolium solution was added to each well and incubated for 30 min. A change in color from yellow to pink indicated the reduction of the dye due to bacterial growth. The MIC was determined for each extract concentration

as the lowest concentration at which no microbial growth was observed, as determined by the absence of color change (Figure 1).

Statistical analysis

Data were analyzed by one-way analysis of variance and the Tukey-Kramer *post hoc* test ($\alpha = 0.05$). Concentrations that inhibited 50% (LC_{50}) and 90% (LC_{90}) of HC egg hatching and motility, as well as those that killed 50% and 90% of larvae, were calculated by a PROBIT analysis using the statistical package SAS 9.0.

RESULTS AND DISCUSSION

Anthelmintic activity

The mean efficacy of the hydroalcoholic extract from *K. daigremontiana* against HC is shown in Table 2. A significantly different egg hatching inhibition rate (EHI%) with respect to positive and negative controls ($P < 0.0001$) was found in the range 100-200 mg mL^{-1} (99.5%). On the other hand, mortality (MOR%) was also significantly different with respect to controls ($P < 0.0001$), being the highest MOR% values observed at 50 and 25 mg mL^{-1} (16.4 and 14.5%, respectively). Finally, significant differences were found among treatments in terms of motility inhibition (IMOT%). The highest IMOT% value was observed at 200 and 100 mg mL^{-1} (85.2 % and 73.1 %, respectively).

As shown in Figures 1 and 2, the LC_{50} and LC_{90} values for egg hatching were 66.5 and 87.3, and 1.5 and 240.9 for motility inhibition in HC L3-stage larvae.

Table 2. Mean efficacy (percentage ± Standard Deviation) of the hydroalcoholic extract from *Kalanchoe daigremontiana* on *H. contortus*

Treatment (mg mL^{-1})	EHI%±SD	IMOT%±SD	MOR%±SD
Water	3.7 ± 0.4^{c}	0^{e}	2.8 ± 1.3^{cd}
KD (200)	99.5 ± 0.94^{a}	85.2 ± 4.2^{b}	6.9 ± 3.1^{c}
KD (100)	99.5 ± 0.95^{a}	73.1 ± 5.3^{c}	13.5 ± 2.6^{b}
KD (50)	10.1 ± 0.88^{b}	64.8 ± 5.7^{cd}	16.4 ± 1.8^{b}
KD (25)	4.7 ± 0.29^{c}	63.0 ± 3.5^{d}	14.5 ± 2.2^{b}
KD (12.5)	3.6 ± 0.30^{c}	59.5 ± 2.1^{d}	13.5 ± 1.1^{b}
Ivermectin 5	100^{a}	100^{a}	100^{a}
SEM	0.019	0.147	0.061
P-value	< 0.0001	< 0.0001	< 0.0001

KD: *Kalanchoe daigremontiana* hydroalcoholic extract. EHI: egg hatching inhibition. IMOT: motility inhibition. MOR: mortality. SEM: standard error of the mean. For each column, different letters indicate significant differences ($\alpha = 0.05$, Tukey test).

Antibacterial activity

As shown in Table 3, *K. daigremontiana* hydroalcoholic extract showed antibacterial activity against both Gram-negative (*P. aeruginosa)* and Gram-positive (*L. monocytogenes, B. subtilis,* and *S. aureus*) bacteria. The MIC was 100 mg mL^{-1} for *P. aeruginosa* and *L. monocytogenes* and 0.781 mg mL^{-1} against *B. subtilis* and *S. aureus.*

Table 3. The minimal inhibitory concentration of *Kalanchoe daigremontiana* hydroalcoholic extract on the Gram (+) and Gram (−) bacteria

Bacterial	Minimal inhibitory concentration (MIC)		
	KD mg mL^{-1}	Kanamycin µg mL^{-1}	Water
E. coli	NA	4	NA
S. typhimurium	NA	4	NA
S. choleraesuis	NA	1	NA
P. aeruginosa	100 [b]	64	NA
L. monocytogenes	100 [b]	2	NA
B. subtilis	0.781[a]	8	NA
S. aureus	0.781[a]	64	NA

KD: *Kalanchoe daigremontiana* hydroalcoholic extract. NA: No activity. Different literals [a,b] in the column indicate significant statistical differences (P≤0.05)

Our results suggest that the *K. daigremontiana* hydroalcoholic extract has a significant activity against *H. contortus* egg hatching *in vitro* (Figure 1). The activity of the extract was as good as that of the positive control (Ivermectin 5 mg mL^{-1}), indicating the feasibility of using this plant species as an anthelmintic agent. Since no significant differences were found between 100 and 200 mg mL^{-1} treatments, the lowest dose could be used to reduce the risk of nematode resistance to the drug. Interestingly, death was not the main effect of the extract, but motility inhibition.

K. daigremontiana hydroalcoholic extract is capable of inhibiting egg hatching and larval motility (Figure 2). Other plant-derived molecules, like flavonoids, flavones, saponins, alkaloids, xanthones (Rivero *et al*., 2016), polyphenols (Akkari *et al*., 2016), tannins (Desrues *et al*., 2016) and pyrazole-5-carboxamide derivatives (Jiao *et al*., 2017), have also been reported to reduce larval motility. The presence in *K. daigremontiana* of flavonoids and polyphenols (Karwatzki *et al*., 1993) has been reported, and it is feasible that these compounds play a role in larval motility inhibition.

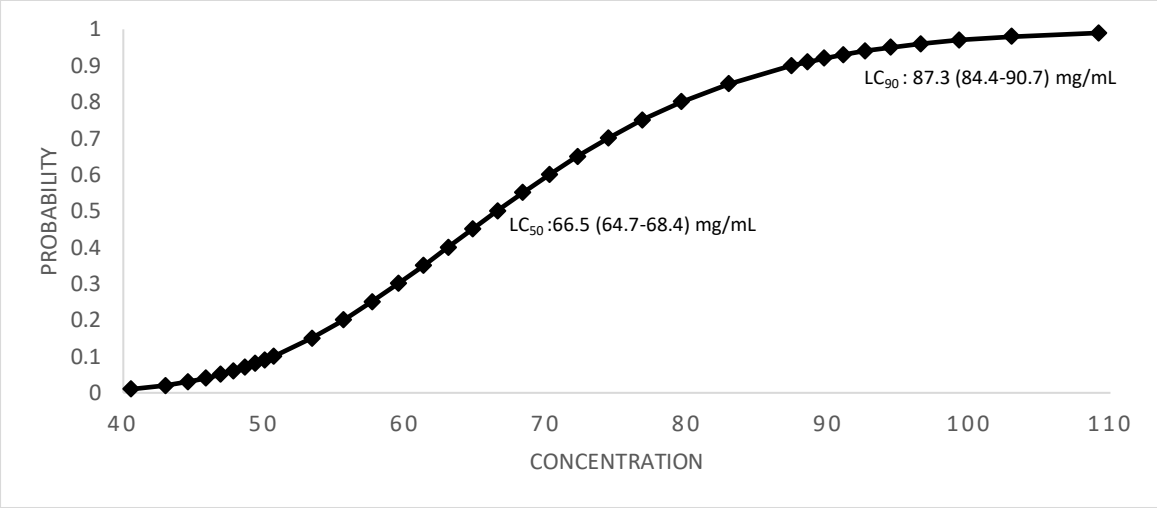

Figure 1. Lethal concentration (LC_{50} and LC_{90}) of *Kalanchoe daigremontiana* hydroalcoholic extract to inhibit the hatching of *Haemonchus contortus* eggs

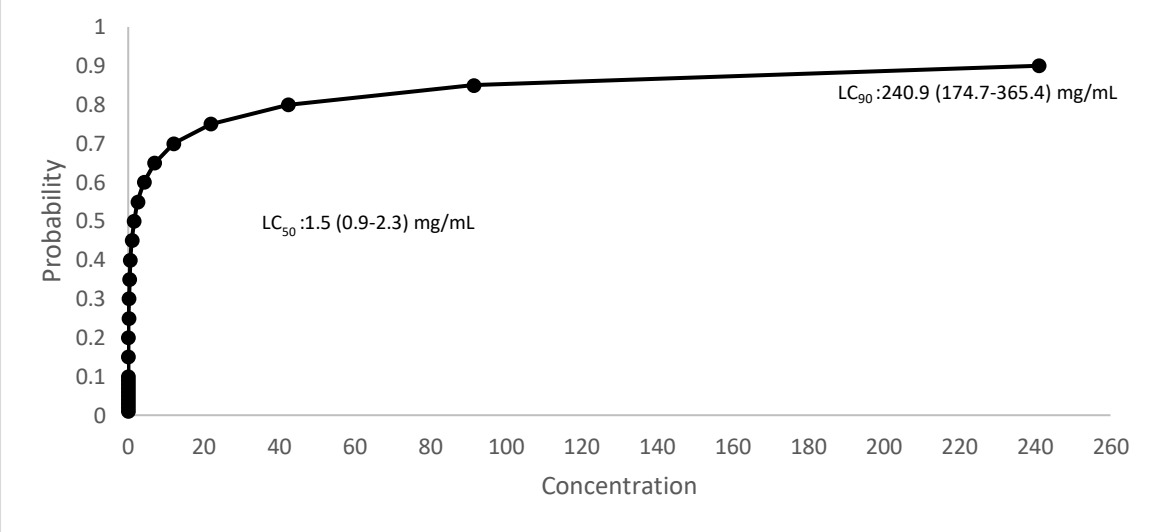

Figure 2. Lethal concentration (LC_{50} and LC_{90}) of *Kalanchoe daigremontiana* hydroalcoholic extract to inhibit motility in *Haemonchus contortus* L3-stage larvae

Our results show that the *K. daigremontiana* hydroalcoholic extract inhibits the motility of HC L3-stage larvae but are not effective in killing them. This could be relevant for the proposed use of *K. daigremontiana* extracts as an anthelmintic, since (Moradpour *et al*., 2013) have described the morphological changes in abomasal tissues due to parasite migration to different parts of sheep abomasa. The results of anthelmintic activity herein reported are similar to those observed by Phatak (2013), who did not find any activity in methanolic and petroleum-ether extracts from *Kalanchoe pinnata* on larval survival; however, the extracts reduced larval motility Unlike that study, however, we also evaluated egg hatch inhibition, finding that 99.5% of eggs did not hatch when exposed to

100 and 200 mg mL^{-1} of the extract, with an LC_{50} of 66.5 mg mL^{-1} and an LC_{90} of 87.3 mg mL^{-1}.According to Lunkad *et al.*, (2016), extracts of species of the subgenus *Bryophyllum*, such as B. *pinnatum*, showed anthelmintic activity in various concentrations (30 and 50 mg/ml) against Indian earthworms *Pheretima posthuman*, counting as antihelmintic activity the paralisis and death of more than 50% of the organisms.

A possible action mechanism of plant secondary metabolites on the eggs and larvae of nematodes like *H. contortus* involves the inhibition or delay of parasite growth and maturation by the affinity of glycoproteins in the cuticle of the parasite to phenolic compounds (mediated by proline residues); polyphenols may bind these proteins, inhibiting parasite motility, feeding, and reproduction, eventually causing their death; additionally, saponins have membranolytic actions (Hernández *et al*., 2018; Irshad *et al*., 2010).

With respect to antibacterial activity, *K. daigremontiana* hydroalcoholic extract showed a higher activity on gram-positive than on gram-negative bacteria; this could be explained considering that gram-negative bacteria have a phospholipidic outer membrane and porins; the phospholipidic membrane that covers structural lipopolysaccharide components make the cell wall impermeable to lipophilic solutes.

Mothana *et al.,* (2009) evaluated 25 plants with antibacterial activity, including *Kalanchoe farinacea.* The methanolic extract of this plant had inhibitory activity on *S. aureus, B. subtilis,* and multi-resistant *Staphylococcus epidermidis* and *S. aureus* at a concentration of 4 mg mL^{-1}, producing 15- and 16-mm inhibition zones on *S. aureus* and multi-resistant *S. epidermidis* and *S. aureus* cultures, respectively, and 10-mm inhibition zones on *B. subtilis* cultures, but had no effect against gram-negative bacteria.

Dichloromethane extracts from *K. pinnata* leaves produced 18-mm inhibition zones when assayed against *E. coli,* but had no effect on *S. aureus* nor *P. aeruginosa.* Those results are in partial agreement with those herein reported. *K. daigremontiana* hydroalcoholic extract had no effect on *E. coli,* but was effective against *P. aeruginosa,* with a MIC of 100 mg mL^{-1}. The same study reported that the methanolic extract contained saponins, cardiac glycosides, and steroids as secondary metabolites with possible antibacterial activity.

Richwagen *et al*., (2019) reported antibacterial activity of extracts from two *Kalanchoe* species, K. *mortagei* and K. *fedtschenkoi*, against ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter cloacae*). Growth inhibition greater than 50% (IC_{50}) was generally observed at concentrations of 256 µg mL^{-1} and 128 µg mL^{-1}.

De Souza Barboza *et al*., (2016), corroborated antimicrobial activity of aqueous extracts of leaves and flavonoids occurring in *Kalanchoe pinnata* (Lam.), concentrations of 100 µg.mL-*1*, showed a growth reduction higher than 50% for *Pseudomonas aeruginosa* and *Citrobacter freundii.*

Akinnibosun *et al*., (1994) reported that an ethanolic extract from *K. pinnata* leaves was more effective against *S. aureus*, *E. coli,* and *P. aeruginosa* than aqueous and methanolic extracts of the same plant, showing inhibition zones of 17.3 ± 1.2, 12.7 ± 0.9, and 8.3 ± 0.9 mm for *S. aureus, E. coli, and P. aeruginosa,* respectively. A qualitative chemical analysis of the ethanolic extract detected flavonoids, steroids, alkaloids, tannins, cardiac glycosides, and reducing sugars, along with secondary metabolites with reported antimicrobial activity, mainly saponins and phenolic compounds like tannins.

CONCLUSION

The present study shows the potential anthelmintic and antibacterial effect of *Kalanchoe daigremontiana* hydroalcoholic extract, against *Haemonchus contortus*, Gram-negative and Gram-positive bacteria, showing the best effect over eggs hatching inhibition and larval motility of *Haemonchus contortus* and on the growth of *B. subtilis* and *S. aureus*. These results indicate that *Kalanchoe daigremontiana* hydroalcoholic extract can be used as alternative natural for control or treatment of diseases associated with these microorganisms. Although the identification of the secondary metabolites associated with these biological activities is necessary, as well *in vitro* and in *vivo* toxicity tests.

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