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Antimicrobial activity of a methanolic extract of *Kalanchoe daigremontiana* Raym. - Hamet and H. Perrier against *Salmonella typhi* ATCC 6589

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Abstract

The increase in the resistance of bacteria to antibiotics represents a priority problem to be addressed for humanity because it causes infectious diseases to become increasingly difficult or impossible to treat, which could lead to a reduction in the hope and quality of life. For this reason, medicinal plants have become a great alternative in the treatment of infections and in reducing bacterial resistance, this through the search for new compounds present in various plant species. *Kalanchoe daigremontiana* is a species of plant from the Crassulaceae family, native to Madagascar. Various studies have shown that this plant has a potential antimicrobial effect against strains of medical importance. The objective of this study was to evaluate the antimicrobial effect of a methanolic extract of *Kalanchoe daigremontiana* against a registered strain of *Salmonella typhi*. A methanolic extract was obtained with a concentration of 1.5 g/mL. The extract had a notable effect against *Salmonella typhi* ATCC 6589 with an inhibition percentage of 41.7% for the concentration used, so it is considered that the *Kalanchoe daigremontiana* extract has the potential to be used as an antibiotic.

Keywords: Antimicrobial; Plant extracts; *Salmonella; Kalanchoe*

1. Introduction

"Kalanchoes" or "calancoes" are tropical and subtropical species native to Madagascar and South Africa, generally considered toxic. They belong to the Crassulaceae family, which is made up of succulent or succulent shrubs and herbaceous plants, included in thirty-five genera. They are characteristic of rocky, hot and dry places. These succulent plants need full sun or partial shade, well-drained soil and little water in the colder months. They are easy to grow, with species with more fleshy leaves needing less water [1].

Kalanchoe daigremontiana, also known by its synonyms as *Bryophyllum daigremontianum* and common names such as devil's backbone, bad mother, mother of thousands, among others, is a perennial herbaceous plant, glabrous, succulent, very robust, with an upright habit, stem simple or little branched, up to one meter high. The leaves are up to 20 cm long, slightly toothed on the margins, have a bright green or tan upper surface, while the underside is grayish green, irregularly spotted with dark green. At the edges of the leaves, between one tooth and the other, adventitious propagules emerge that, when they fall to the ground, create new small plants that take root. Its flowers are tubular red-orange in color that are grouped in an umbel-type inflorescence and measure up to 2 cm long. They bloom at the end of winter in adult plants. This plant can reproduce by forming a multitude of small helical-shaped seedlings, on the edge of each of the fleshy and triangular leaves; when these seedlings fall, they effectively root even in small portions of soil [2].

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Kalanchoe daigremontiana is a naturalized plant in many parts of tropical and subtropical Africa, Asia (Indian Ocean islands), North America, South Africa and can also be found in Bangladesh [3]. Due to its invasive characteristics, it inhibits the repopulation of native plants and modifies soil biology in semi-arid areas [4]; even so, *K. daigremontiana* has been introduced to numerous tropical and subtropical regions: Florida, Puerto Rico, the Pacific Islands, Australia, India, China and the Canary Islands. It is widely cultivated in the gardens of several tropical countries. It is well established and considered an aggressive naturalized weed almost everywhere and is usually found along roadsides. Therefore, it is a plant without problems for cultivation [1].

In traditional medicine, medicinal plants have played a fundamental role as a means to cure diseases in people. Currently, communities, especially rural ones, use them by accumulating ancestral practices of selection, management and conservation of knowledge that they have transmitted from one generation to another. This information has been important for the discovery of different medicines that we use today made from plants [5]. Among their various uses in medicine, plants have been used in the treatment of infectious diseases and currently represent an alternative to the inconveniences and adverse effects generated by the improper use of antibiotics. The World Health Organization (WHO), in 2001, reported that 80% of the world's population uses traditional knowledge to treat their diseases due to the properties and effective results that medicinal plants present in infectious diseases. In this sense, it has been shown that plants are a potential source of active ingredients for the development of new drugs and that they have a positive effect against a wide range of diseases, since they synthesize compounds with important biological activities, such as, for example. For example, antimicrobials [6].

Several *Kalanchoe* species (*K. pinnata*, *K. daigremontiana*, *K. brasiliensis*) have shown various antitumor, antihistamine, anti-inflammatory and immunomodulatory activities. *K. daigremontiana* has been used in traditional medicine to combat various infections, as an anti-inflammatory and to treat various types of cancer, especially in indigenous peoples of Africa and Brazil [4]. Rivero *et al.* [7] used five different concentrations (12.5 to 200.0 mg/mL) of an extract of *Kalanchoe daigremontiana* leaves and stems to determine its anthelmintic and antibacterial effect in vitro. The determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) allowed the evaluation of the antibacterial activity of the extract on various strains (not registered) such as: *Listeria monocytogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. The extract showed activity on Gram positive and Gram negative bacteria, determining an MIC of 100 mg/mL on *Pseudomonas aeruginosa* and *Listeria monocytogenes* and 0.781 mg/mL for *Bacillus subtilis* and *Staphylococcus aureus*. These results indicate that the hydroalcoholic extract of *Kalanchoe daigremontiana* has potential anthelmintic and antibacterial effects and that it could be used as a biological control strategy and as an antimicrobial.

2. Materials and Methods

Kalanchoe daigremontiana plants were obtained from the Cuemanco plant and flower market in the Xochimilco mayor's office in Mexico City. For their identification, the plants were compared with images available online of the species and keys were also used for the identification of species of the genus Kalanchoe from Hurrel *et al.* [8].

For the preparation of the methanolic extracts of the *Kalanchoe daigremontiana* plant, 10 grams of leaves were weighed and crushed. The extraction was carried out at room temperature with methanol and acetic acid; for each gram of biomass, 60 mL of methanol and 2 mL of acetic acid were used. It was left to rest for 48 hours. Then the solvent was evaporated at a temperature of 40 °C. Finally, it was resuspended in methanol and stored at 4 °C.

Evaluation of the antibacterial activity of the extract. In most cases, microbiological assays depend on the microorganisms present in the culture being typical, reproducible and capable of maintaining their physiological and morphological characteristics. To help control this, quality control standard strains are essential in the validation process. There are several strains that are used for quality control during antimicrobial assays, such is the case of the American Type Culture Collection (ATCC), which is a non-profit organization dedicated to the conservation, distribution and verification of microbial strains and cell phones for research and use in academia, government and industry [9]. It is considered one of the largest and most respected collections of microbial strains in the world and is a valuable resource for research in various areas, including biotechnology, public health and the pharmaceutical industry. ATCC offers a wide variety of bacterial, fungal, viral, cellular and genomic strains that are used as quality controls in the research and development of new medical products and treatments [10].

The bacterial strain was adjusted with an isotonic saline solution to tube 1 of the Mac Farland nephelometer. After adjusting, a 1:10 dilution of that tube was made to obtain a concentration of $3x10^7$UFC / mL, they were inoculated on Müller-Hinton agar plates. Afterwards 3 sterile filter paper discs of 7 mm diameter impregnated with 30µL of the test extracts, one with 10 µL chloramphenicol (5mg/mL) and another with 30µL of methanol were placed; ten plates were

made for each strain and were incubated for 24 h at 37 ° C. The inhibition halos formed around the discs were measured [11].

The inhibitory effect was calculated using the following equation [12]:

$$\%\ inhibitory\ effect = \frac{average\ inhibition\ zone}{diameter\ of\ the\ inhibition\ zone\ of\ positive\ control} \times 100$$

3. Results and discussion

Figure 1 shows the inhibition halos formed, which on average measured 23.3 mm, so the percentage of inhibition shown was 41.7%. The *Salmonella typhi* ATCC 6589 strain, as a Gram-negative bacteria, has a more complex cell wall structure than that of Gram-positive bacteria, as it has a thin layer of peptidoglycan and an outer membrane composed of lipopolysaccharides (LPS) [13]; furthermore, Gram-negative bacteria often produce enzymes, such as β-lactamases, that can inactivate certain antibiotics, such as penicillins and cephalosporins [14]. Therefore, the methanolic extract of *Kalanchoe daigremontiana* may be acting on one or both pathways: crossing the outer membrane composed of lipopolysaccharides or preventing the synthesis of β-lactamases enzymes.

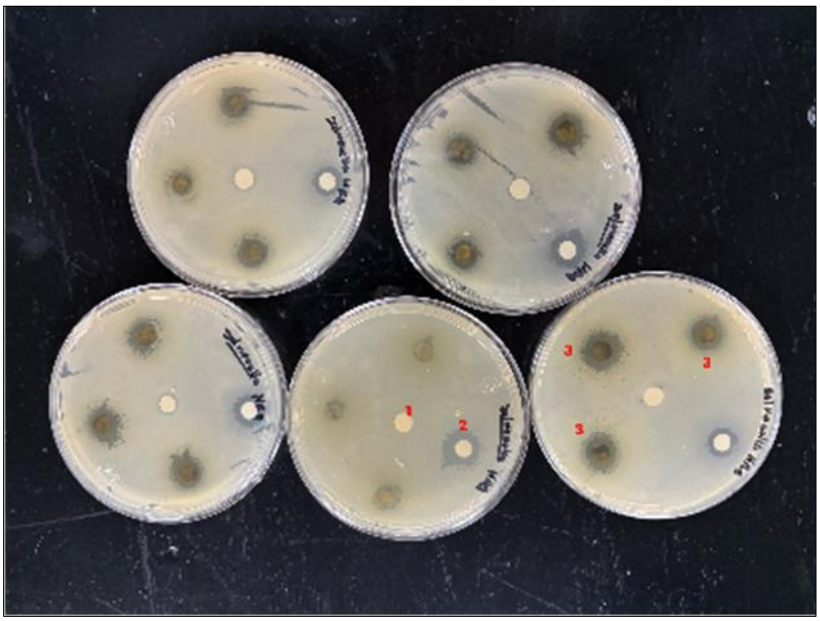

Figure 1 Inhibition of the growth of *Salmonella typhi* ATCC 6589 by methanolic extract of *Kalanchoe daigremontiana.* 1) Methanol, 2) Chloramphenicol, 3) Methanolic extract

4. Conclusion

These results reflect the potential use of the extract as an antibiotic; however, it is important to highlight that most of the studies carried out so far are in vitro and more work is needed to fully understand the mechanism of action and effectiveness of *Kalanchoe daigremontiana* compounds against specific microorganisms.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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