

**DETERMINATION OF ANTIMICROBIAL ACTIVITY IN LEAVES AND FLOWERS OF
CHROMOLAENA SCABRA (L. F.) R.M. KING AND H. ROB.****PAULA ALEJANDRA GIRALDO VILLAMIL¹, ANDRÉS CAMILO ANDRADE BURBANO¹, LUIS POMBO OSPINA²,
JANETH ARIAS PALACIOS³, ÓSCAR EDUARDO RODRÍGUEZ AGUIRRE^{4*}**

¹Department of Chemical Pharmaceutical Program, Universidad de Ciencias Aplicadas y Ambientales U.D.C.A., Bogotá Colombia. ²Centro de Investigación, Fundación Universitaria Juan N. Corpas, Bogotá Colombia. ³Department of Microbiology, Industrial Microbiology Program, Industrial Environmental and Biotechnology Group, Pontificia Universidad Javeriana, Bogotá, Colombia. ⁴Environmental Engineering Program, Faculty of Engineering, Research Group CHOC-IZONE, Universidad El Bosque, Bogotá, Colombia.
Email: rodriguezoscare@unbosque.edu.co

Received: 23 June 2020, Revised and Accepted: 06 August 2020

ABSTRACT

Objective: The objective of the study was to determine the antimicrobial activity of leaf and flower extract in *Chromolaena scabra* (L. f.) R.M. King and H. Rob., against selected strains of bacteria and fungi.

Methods: The agar diffusion method with plate perforation was developed; the microorganisms used were strains of *Staphylococcus aureus* and *Escherichia coli*, *Aspergillus niger*, and *Penicillium digitatum*. Rifampicin was used as a positive control. The evaluation was performed by measuring the diameter of the growth inhibition zones around the holes. The inhibitory effect of the plant extracts was obtained by its efficiency compared to the positive control. A comparison with fluconazole and ketoconazole was performed to determine how much of the extract is required to cause inhibition of fungal growth from the standard.

Results: IC₅₀ was determined by relating the ln of mass evaluated with respect to the square of the inhibition halo; ethanolic extracts of leaves and flowers of petroleum ether with IC₅₀ values of 85.8 mg/ml and 50.3 mg/ml showed the highest inhibitory effect against *S. aureus*; the extract of petroleum ether and ethanol from leaves with IC₅₀ of 64 mg/ml and 60 mg/ml, respectively. They were effective with *A. niger*. Leaf petroleum ether extract showed the best relative antifungal activity against *A. niger* with respect to fluconazole equivalent to 459.51 when fluconazole is 1.0.

Conclusion: The extracts with high potential to inhibit the growth of microorganisms were determined to be ether flowers of petroleum and ethanol leaf extracts.

Keywords: Agar diffusion method, *Chromolaena scabra*, antimicrobial activity, Soxhlet extraction, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium digitatum*.

© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2020.v13i9.38835>

INTRODUCTION

Plants are usually known to make use of their diverse health benefits through the numerous phytochemicals they contain. Plant-derived substances have recently turned to be of great interest due to their versatile applications. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [1]. The development of antibiotic resistance in microorganisms is a global challenge for the clinicians, pharmacist, and research scientists leading to the development of new medicinal formulations that are effective and easily consumable [2]. Medicinal plants play a significant role in providing primary, health-care services to rural people and are used by about 80% of the marginal communities in the world [3]. Antimicrobial resistance is very common. Bacteria and fungi continue to develop drug resistance by employing various mechanisms to survive in the lethal environment created by antimicrobials [4]. It is now the requirement of developing the alternative drug line to treat infectious diseases caused by microorganisms such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. [5].

The primary metabolites present in plant species are carbohydrates, amino acids, fatty acids, nucleic acids, amines, chlorophylls, and metabolic intermediaries of the anabolic and catabolic pathways, which, in turn, produce secondary metabolites, which are substances that are not directly involved in growth or development, that is, substances that are not necessary for an organism to exist as such, but simply provide

the individual who produces them with an advantage in responding to environmental stimuli, either as a defense mechanism against predators or as storage. In 2000, researchers at the University of Port Harcourt, Faculty of Science, Department of Biochemistry, Nigeria, Igboh *et al.* identified the chemical profile of *Chromolaena odorata* [6].

According to Taleb-Contini, all the crude extracts (ethanolic and dichloromethanic) from leaves and stems of *Chromolaena squalida* and leaves and flowers from *Chromolaena hirsute* showed antimicrobial activity, mainly against Gram-positive (*Staphylococcus* and *Streptococcus*) bacteria, at 1000 mg/mL [7]. The genus *Chromolaena* belongs to Eupatorieae tribe and Praxelinae subtribe (King and Robinson, 1987) and comprises about 170 species distributed between sea level and 3900 m of altitude and from the south of United States to south of Argentina, with the highest concentration of species in Brazil. Among the species that stand out for their wide distribution are *Crataegus laevigata*, *Chromolaena ivifolia*, and *C. odorata*, the latter being the only species introduced to Africa and Asia [8]. This research aimed to determine that the extracts of leaves and flowers of *Chromolaena scabra* (L.f.) R.M. King and H. Rob. present antimicrobial activity, thus responding to the activity observed in other species belonging to the same genus.

METHODOLOGY**Extraction**

The extraction process was started with previously dried and ground plant material, having an initial amount of 600 g of leaves and 400 g

of dried ground flowers of *C. scabra* (L. f.) R.M. King and H. Rob., this plant material was entered into the Soxhlet equipment, where the solid-liquid extraction was carried out during 8 days, at boiling temperature according to the solvent (petroleum ether, dichloromethane, and ethanol) and, when the corresponding cycles of this process were completed, the extracts were passed to be concentrated in the rotavaporator at low pressure, temperature of 40°C, and a revolution of 60 rpm, where the solvent was recovered and the desired extract was obtained. Extracts were concentrated under reduced pressure and their dry weight was obtained.

Antimicrobial activity test

The microorganisms used in antibacterial activity determination in this study come from the so-called control strains, according to the classification of the American Type Culture Collection (ATCC). For Gram-positive bacteria, *S. aureus* (ATCC 6538) was used as indicator microorganism; and for Gram-negative, *E. coli* (ATCC 8739) was used.

The gel diffusion method by perforation in plate was used, using Mueller-Hinton agar as bacterial growth medium. Thirty-four grams of agar were weighed, then diluted in 1.0 L of sterile water, and the mixture plus the other instruments were sent to autoclave for 1 h [9-11].

For inoculum preparation, 8–10 colonies of each microorganism were transferred with a bacteriological handle, to a tube with 3–4 mL with 0.85% w/v saline, forming a bacterial suspension in the broth, which should have a similar turbidity to that of standard 0.5 in the McFarland nephelometer (1.5×10^8 ufc/mL). The inoculum obtained by this procedure was massively seeded with swabs in Petri dishes with the previously prepared Mueller-Hinton agar, which was then perforated making 4 wells for evaluation extract.

The antibiotic rifampicin, prepared with 1 mg/2 mL dimethyl sulfoxide (DMSO) (equivalent to 0.5 mg/mL DMSO), was used as a positive control for *S. aureus* assay. To evaluate the activity volumes of 10, 20, 30, and 50 μ l, 10 μ l (0.005 mg/ml), 20 μ l (0.01 mg/ml), 30 μ l (0.015 mg/ml), and 50 μ l (0.025 mg/ml) were used for the test. To prepare the samples, 200 mg of extract were taken and dissolved in 1 ml of DMSO (200 mg/mL DMSO); to evaluate the activity volumes of 20, 30, 40, and 60 μ l, 20 μ l of respective extract equivalent to 4 mg/ml, 30 μ l of respective extract equivalent to 6 mg/ml, 40 μ l of respective extract equivalent to 8 mg/ml, and 60 μ l of respective extract equivalent to 12 mg/ml.

Once the controls and the respective samples were seeded, the Petri dishes were incubated at 37°C, during a period of 24 h, having the same incubation time for all the dishes. The halos were measured at the end of the incubation period.

Antifungal activity test

The microorganisms used in the determination of antifungal activity in this study come from the so-called control strains, according to the classification of the ATCC. For *Aspergillus niger* and *Penicillium digitatum* fungi, the gel diffusion method by perforation in plate was used, using potato dextrose agar (PDA) agar (dextrose potato) as growth medium. Thirty-nine grams were weighed and diluted in 1.0 L of sterile water, and the mixture plus the other instruments were sent to autoclave for 1 h.

For inoculum preparation, a part of the fungus culture was taken and transferred to a tube with 3–4 mL with 0.85% w/v saline solution. The inoculum obtained by this procedure was massively seeded with swabs in Petri dishes with previously prepared PDA agar, which was then perforated making 1 well for the evaluation of the extract.

As positive control of the assay performed with *A. niger* and *P. digitatum*, the antifungal fluconazole and ketoconazole were used, which was prepared using 2 mg dissolved in 5 mL of DMSO equivalent to 0.4 mg/mL DMSO, 10 μ l (0.004 mg/ml), 20 μ l (0.008 mg/ml), 30 μ l

(0.012 mg/mlmg/ml), and 40 μ l (0.016 mg/ml) which were used in the wells.

The activity of leaf and flower extracts obtained with increasing polarity solvent (petroleum ether, dichloromethane, and ethanol) was evaluated, these were prepared with 200 mg dissolved in 1 mL DMSO (200 mg/mL DMSO), volumes of 50 μ l (10 mg/ml) and 100 μ l were evaluated (20 mg/ml). Each concentration was added in a Petri dish.

Once the controls and the respective samples were seeded, the Petri dishes were incubated at 22°C, during a period of 10 days, having the same incubation time for all the dishes. The halos were measured at the end of the incubation period.

RESULTS

The IC_{50} was determined by graphing the ln of the evaluated mass with respect to the square of the inhibition halo, where on the graph of the line $y = mx + b$, where $y = \ln 50$ and $x = IC_{50}$; as shown in Fig. 1, the extracts with the best activity are the ethanolic extract of leaves with a critical mass of 85.8 and the flower petroleum ether with a critical mass of 50.3. This means that these extracts have a significant concentration for the inhibition of the microorganism under study. It should be taken into account that they are extracts and they have many metabolites in low concentration in their composition, so they have activity.

As shown in Fig. 2, the relative antibacterial activity (ABR) obtained by dividing the IC_{50} of the sample by IC_{50} of the standard was determined. The extracts that have a greater activity against *S. aureus* microorganism were the ethanol extract of leaves with an ABR equivalent to 197.5, followed by the ether extract of efflorescence with an ABR 336.7 when the ABR of rifampicin is equivalent to 1.0, this comparison was made to determine what amount of extract that has more than 500 compounds in different proportions is required to cause the inhibition of the bacterial growth of the microorganism equivalent to that of the standard of a pure molecule of which less amount is required because it is a pure substance with specific use for the treatment of *S. aureus*.

Comparing the data obtained in this investigation with García Rodríguez, 2000, who expressed that inhibition halos of 16 mm against rifampicin indicate a good level of growth inhibition in *S. aureus* strains and inhibition halos greater than 20 mm, are indicative of sensitivity. In addition, rifampin concentrations of 5 mg are expressed as corresponding to inhibition halos of 34 mm.

For extracts of *C. scabra* leaves, bacterial growth inhibition data are obtained in a range of 18–25 mm with petroleum ether, ethanol from 7 mm to 17 mm, and with dichloromethane from 30 mm to 43 mm, resulting in these extracts presenting metabolites in a concentration that has intermediate and sensitive capacity for inhibition of growth of the microorganism under study. For flower extracts of the *C. scabra*, data on inhibition of bacterial growth in ranges of 23 mm–36 mm are obtained with petroleum ether, ethanol from 11 mm to 17 mm, and dichloromethane from 27 mm to 32 mm, these halo values of inhibition are equivalent to an intermediate inhibitory activity for the microorganism under study.

This study was performed with *E. coli* to determine growth inhibition but no results were obtained from the extracts of leaves and flowers of *C. scabra* (L.f.) R.M King and H. Rob. against this microorganism.

As shown in Fig. 3, against *A. niger*, the best IC_{50} is obtained from the petroleum ether extract from leaves with a critical mass of 64 and the ethanol extract from leaves with a critical mass of 60, this means that these extracts have a significant concentration for the inhibition of the microorganism under study.

As shown in Fig. 4, against *P. digitatum*, the best IC_{50} is those obtained from the ether extract of oil flowers with a critical mass of 190 and the dichloromethane extract of flowers with a critical mass of 120, this

means that these extracts they have a significant concentration for the inhibition of the microorganism under study.

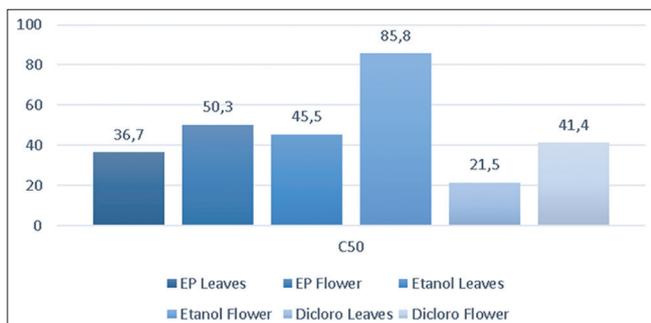


Fig. 1: IC₅₀ leaves and flower extract from *Chromolaena scabra* against *Staphylococcus aureus*

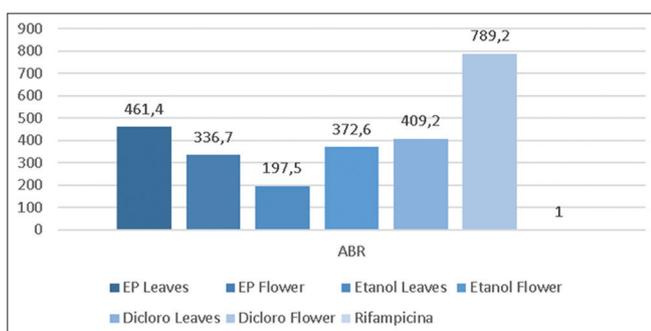


Fig. 2: Relative bacterial activity of leaves and flower extracts of *Chromolaena scabra*, with respect to the rifampicin pattern against *S. aureus*

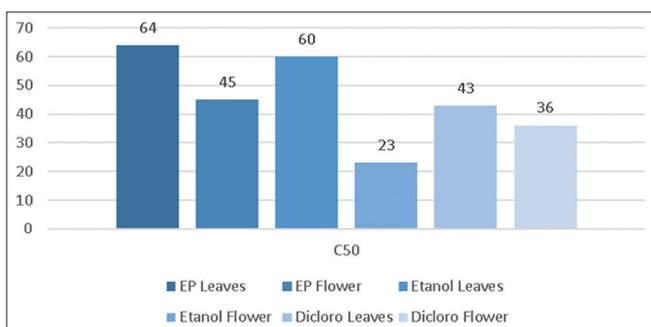


Fig. 3: C₅₀ leaves and flower extract from *Chromolaena scabra* against *Aspergillus niger*

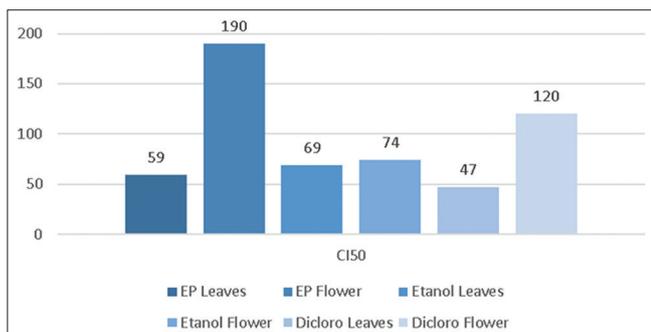


Fig. 4: C₅₀ leaves and flower extract from *Chromolaena scabra* against *Penicillium digitatum*

In Fig. 5, we observe the relative antifungal activity (AFR), with respect to fluconazole and ketoconazole, where the ether extract of leaf oil has the best AFR against the microorganism *A. niger*, equivalent to 459.51 followed by the ethanol extract of leaves with an AFR of 490.14 when the AFR of fluconazole is 1.0. When comparing the extracts with ketoconazole, we see that the petroleum ether extract of the leaves has the best AFR against *A. niger* microorganism, equivalent to 520.9 followed by the ethanol extract of the leaves with an AFR of 555.6 when the AFR of ketoconazole is 1. Comparisons are made to determine how much of the extract that has more than 500 compounds in different proportions is required to cause the inhibition of the bacterial growth of the microorganism equivalent to the standard of a pure molecule of which less amount is required because it is a pure substance with specific use as an antimicrobial.

Fig. 6: Relative fungal activity of leaf and flower extract of *Chromolaena Scabra* against *Penicillium digitatum* in presence of fluconazole and ketoconazole

In Fig. 6, we observe the relative antifungal activity (AFR), with respect to fluconazole and ketoconazole, where the ether extract of flower oil has the best AFR against the microorganism *P. digitatum*, equivalent to 87.72 followed by the dichloromethane extract of flowers with an AFR of 138.9 when the AFR of fluconazole is 1. When comparing the extracts with ketoconazole, we see that the petroleum ether extract of the flowers has the best AFR against *P. digitatum* microorganism, equivalent to 941.18 followed by the dichloromethane extract of flowers with an AFR of 1490.2 when the AFR of ketoconazole is 1. Comparisons are made to determine how much of the extract that has more than 500 compounds in different proportions is required to cause the inhibition of the bacterial growth of the microorganism equivalent to the standard of a pure molecule of which less amount is required because it is a substance pure with specific use as an antimicrobial.

DISCUSSION

According to the literature, the antifungal agents used for this work do not present inhibition for *A. niger* and *P. digitatum* growth; however, the results show inhibition for these two fungi. The extract of leaves in petroleum ether shows inhibition against *A. niger* with halos of 40 and 46 mm, in ethanol halos of 16 and 27 mm, and with dichloromethane of 19 and 25 mm. The inhibition halos of *C. scabra* flower extracts against *A. niger* were 21 mm and 27 mm with petroleum ether, with ethanol 11 and 14 mm, and dichloromethane 20 and 24 mm.

For *P. digitatum* growth inhibition, halo for petroleum ether was 31 and 38 mm, ethanol 21 and 33 mm, and dichloromethane 31 and 35 mm. Moreover, lower extract growth inhibition for *P. digitatum* was 26 and 67 mm with petroleum ether extract, for ethanol 46 and 63 mm, and dichloromethane 40 and 60 mm.

References [12,13] report that fluconazole and ketoconazole antifungal exhibit low growth inhibition against fungi such as *A. niger* and *P. digitatum* in contrast, our work shows that these antifungals present significant growth inhibition against the fungi used for this work.

It is determined that the flower and leaf extracts in ethanol have a higher C₅₀ for *S. aureus* growth inhibition compared to the other different extracts.

It is determined that growth inhibition of *S. aureus* compared to rifampicin is higher in the leaf extracts in ethanol than in the other extracts.

Comparing literature with results obtained for growth inhibition of *S. aureus*, it is concluded that this microorganism is sensitive to different extracts used in this test, achieving inhibition halos against bacterial growth. These results agree with Catalán and Montejo [14] which describe antibacterial activity and inhibitory effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *K. pneumoniae*. The tests that were carried

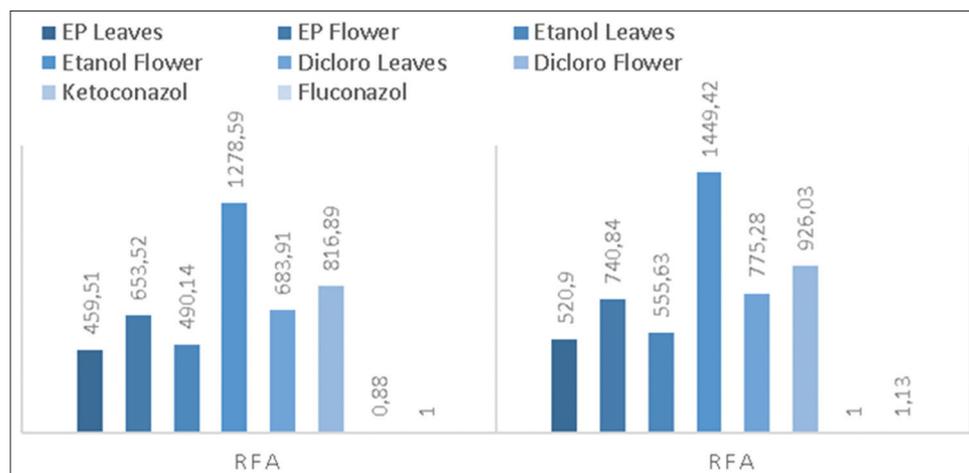


Fig. 5: Relative fungal activity of leaves and flower extracts of *Chromolaena Scabra* and fluconazole and ketoconazole as standard against *A. niger*

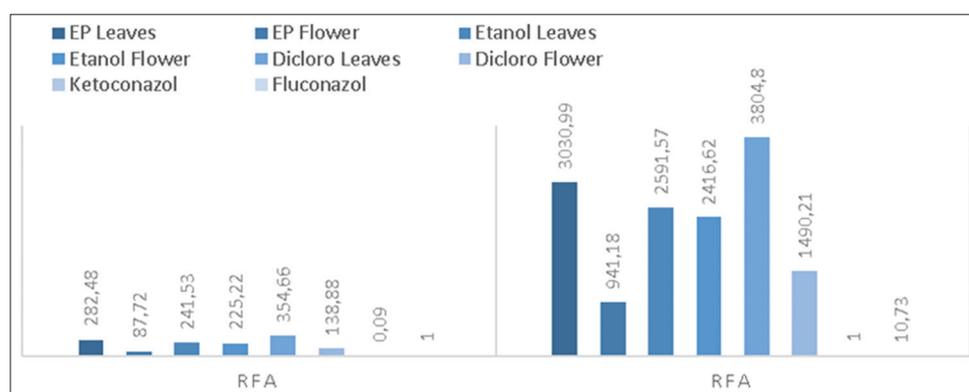


Fig. 6: Relative fungal activity of leaf and flower extract of *Chromolaena Scabra* against *Penicillium digitatum* in presence of fluconazole and ketoconazole

out to determine the bacterial activity of the extracts against *E. coli* did not show significant inhibition for this microorganism.

CONCLUSION

The result of the present study suggests for further investigation that the best results for inhibiting the growth of fungi's were petroleum flowers and leaves extracts. Growth of the *S. aureus* strain was strongly inhibited by ethanol and petroleum ether extracts. Those extracts do not present any significant inhibition to *E. coli* strain.

ACKNOWLEDGMENT/FUNDINGS

The authors are thankful to Javeriana University, Juan N Corpas Foundation and Labfarve Laboratories for support this research.

AUTHORS' CONTRIBUTIONS

All authors had equally contributed with this research work.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Arunkumar K, Chandrashekar KR. Phytochemical evaluation and *in vitro* antimicrobial and antioxidant studies of leaf and stem bark extracts of *Polyalthia fragrans* (Dalz.) Bedd. An endemic species of Western Ghats. *International J Pharm Pharm Sci* 2017;9:20-4.
2. Pandey S. Antibacterial and antifungal activities of *Ocimum gratissimum* L. *Int J Pharm Pharm Sci* 2017;9:26-31.
3. Pruthvi ML, Mahesh MK, Rohini SM. Efficacy of *Euphorbia heterophylla* Latex against pathogenic bacteria and fungi. *Asian J Pharm Clin Res* 2020;1:141-5.
4. Sekyere JO, Asante J. Emerging mechanisms of antimicrobial resistance in bacteria and fungi: Advances in the era of genomics. *Future Microbiol* 2018;13:241-62.
5. Amita SR, Shobha KL, Manjunath SH, Sreedhara PK. *In vitro* antibacterial and antifungal activities of aqueous and ethanolic leaf extracts of *Acacia auriculiformis*. *Asian J Pharm Clin Res* 2018;11:480-2.
6. Igboh MN, Ikewuchi CJ, Catherine C. Chemical profile of *Chromolaena odorata* L. (King and Robinson) leaves. *Pak J Nutr* 2009;8:521-4.
7. Taleb-Contini SH, Salvador MJ, Watanabe E, Ito IY, Oliveira CR. Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* Species. *Rev Bras Ciênc Farma* 2003;39:403-8.
8. Díaz-Piedrahita S, Rodríguez-Cabeza BV. Novedades en asteráceas colombianas-I. *Rev Acad Colomb Cienc* 2011;35:411-24.
9. Cole MD. Key antifungal, antibacterial and anti-insect assays-a critical review. *Biochem Syst Ecol Oxf* 1994;22:837-56.
10. Grove DC, Randall WA. *Assay Methods of Antibiotics: A Laboratory Manual [Antibiotics Monographs]*. New York: Medical Encyclopedia Inc.; 1955. p. 80.
11. Narváez M, Rodríguez OE, Arias J. Antimicrobial activity of *Hymenaea courbaril* L. Fruit. *Asian J Pharm Clin Res* 2020;13:200-3.
12. Cuenca M, Medallo E, Rodríguez JL. Importancia clínica de los mecanismos de resistencia de los hongos filamentosos a los antifúngicos. *Unidad Micol* 2002;20:523-30.
13. Adewumi AM, Olusola-Makinde O, Kolawole OM. Evaluation of phytochemical constituents and antibacterial activity of *Chromolaena odorata* L. Leaf extract against selected multidrug resistant bacteria isolated from wounds. *South Asian J Res Microbiol* 2020;5:1-9.
14. Catalán M, Montejo JC. Antifúngicos sistémicos. Farmacodinamia y farmacocinética. *Rev Iberoam Micol* 2006;23:39-49.