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## RESEARCH ARTICLE

# ANTIMICROBIAL ACTIVITY OF EXTRACTS OF RED MANGLE BARK, GARLIC CLOVE, ONION AND LEMON PEEL

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**ABSTRACT**

In Mexico, the main causal agents of hospital infections are the species of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*; whose strains are usually multiresistant to antibiotics. In view of this situation, new antimicrobials are required, with structures and mechanisms of action different from current commercial antibiotics and, in the future, new drugs to counteract the infections caused by these microorganisms. The foregoing has led to the investigation of new antimicrobial substances from plants considered popular medicinal. These plants have a wide range of chemicals, whose active ingredients can be useful as a medicine and can be found in their entire structure or only in some sections. Therefore, this work evaluated in vitro the bactericidal activity of ethanolic extracts from red mangle bark, garlic clove, onion and lemon peel in multi-resistant gram-negative bacteria.

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## INTRODUCTION

Currently, the therapeutic effectiveness of numerous commercial antibiotics against multi-resistant pathogenic bacteria is decreasing (Baz, 2018; Núñez, 2018). Therefore, numerous investigations are focused on the search for new biological compounds from medicinal plants with antimicrobial activity, giving the opportunity to find new active pharmacological agents (Casanova, 2012). Red mangrove (*Rhizophora mangle* L.) is a perennial tree or shrub, halophyte, from 1.5 to 15 m (up to 30 m) in height with a diameter at chest height up to 50 cm. The decoction of its leaves is used in the treatment of poisoning by contaminated fish and ulcers.

The extract of red mangrove bark suppresses the secretions produced by superficial scarring. They are also used to mitigate respiratory and digestive ailments, venereal diseases and infections of the skin and leprosy (Regalado, 2016). Garlic (*Allium sativum* L.) consists of a bulb (garlic head), which is formed by a variable number of bulbs (teeth) inserted on a crushed axis. Garlic extracts have pharmacological properties attributed mainly to their sulfur components and used by the population for the treatment of intestinal parasites, auditory infections, defects or deficiencies of hemostasis, dyslipidemia and hypotensive activity among other conditions (Chalar, 2014; Ramírez, 2016). Onion (*Allium cepa*) is a generally round white, yellow, or red bulb, with a content of fructans and organ sulfur compounds, which benefit the gastrointestinal

functions, increasing the resistance to colonization of the intestinal mucosa by pathogenic microorganisms, reduce cholesterol and triglycerides in the blood and inhibit platelet aggregation (Dávila, 2013). Lemon (*Citrus latifolia*) is an important medicinal plant whose raw extracts from different parts of the plant contain flavonoids, with medicinal uses such as: antifungal, antineoplastic and antiviral mainly. Likewise, the extracts of the citrus peel are a rich source of flavonoid glycosides, coumarins and sitosterol, glycosides and volatile oils with antimicrobial activity (Argote, 2016). For the aforementioned, this work was carried out in order to evaluate the antimicrobial activity of ethanolic extracts of the red mangle bark, garlic clove or onion or lemon peel in multi-resistant gram-negative bacteria.

## MATERIALS AND METHODS

**Identification and antimicrobial susceptibility of the bacteria inoculated in the study:** All wild strains included in this work come from a collection of strains isolated from hospitalized patients donated to the Laboratory of Microbiology of the Center for Biomedical Research of the UAC. The identification and determination of the antimicrobial susceptibility of the strains were carried out through automated microbiological system VITEK II.

**Technique for obtaining ethanolic extracts:** The extracts were obtained using a Soxhlet extraction equipment, using 96% ethanol as a solvent. Extraction consisted basically of the successive washing of a solid mixture with a specific solvent, which "flushes or extracts" the most soluble components from the mixture. So, the bulbs of garlic or anion, bark of mangle and lemon peel were used to make a cartridge with filter paper, which was placed in the extractor. Then a ball flask with ethanol as solvent was placed in the lower part of the extractor. The water inlet and outlet hoses were positioned to the refrigerant. Once the Soxhlet equipment was assembled, it was heated to a temperature of 78 °C during 5 to 8 hours for the total extraction of the soluble components (Casanova, 2012).

**Preparation of Tripticasein Soy Agar with the ethanolic extract for MIC's:** Soya Tripticasein Agar at pH 7.0 was added the concentration of the extract to be evaluated (STAE) to determine the Minimum Inhibitory Concentration (MIC). Then the STA enriched with extract was poured into Petri dishes until its solidification. Subsequently, the petri dishes were incubated at 35 °C for 24 hours in a bacteriological stove as sterility control (Casanova, 2012).

**Inoculum preparation method:** Two or three colonies with similar morphology of Soya Tripticasein Agar were harvested to transfer them to a tube with 3 mL of saline solution. Then, the bacterial suspension was incubated at 35 °C until reaching the standard turbidity of 0.5 of the McFarland scale, which is equivalent to 1 to 2 X $10^8$ CFU/mL. This technique was used for each of the bacterial strains (Casanova, 2012).

**Inoculation by the Kirby-Bauer method on ASTE:** The inoculation was made within 15 minutes after adjusting the turbidity of the bacterial suspension to 0.5 of the McFarland scale. After a cotton swab was immersed in the tube with the bacterial suspension and it was rotated several times pressing firmly against the inner wall of the tube above the liquid level to remove excess inoculum. The inoculation was carried out according to the method of Kirby-Bauer, on the entire surface of the agar and without forgetting to pass the cotton swap on the edges of Petri dish. Subsequently, the inoculated plates were incubated at 35 °C for 72 hours, to demonstrate that the extracts used in the test do have a bactericidal effect and not a bacteriostatic effect (Casanova, 2012).

**Determination of the minimum inhibitory concentration (MIC):** To determine the MIC of the ethanolic extracts of the natural products mentioned above (necessary to kill the bacteria evaluated in this work), the agar dilution method was used. The MIC was defined as the lowest concentration of extract in which the tested bacteria was not showed visible growth compared to the control during the incubation time. To carry out the tests, STAE plates with different concentrations of the extracts were inoculated, with the bacterial suspension adjusted to 0.5 McFarland. Then Petri dishes were incubated at 35 °C for 72 hours in a bacteriological stove (Casanova, 2012).

**Interpretation of the bactericidal effect:** After the incubation process, the presence or absence of bacterial growth in the cultured Petri dishes was determined. If there was growth, it meant that the ethanolic extract of red mangrove bark or clove of garlic or onion or lemon peel did not kill the bacteria in the concentration of the evaluated extract; but if the bacteria did not grow, then, it was interpreted as an extract with a bactericidal effect (Casanova, 2012).

## RESULTS

**Bacterial resistance:** This work included 30 wild strains of each of the following species: *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. All bacteria were resistant to the antibiotics commonly used in hospital therapy. So, strains of *P. aeruginosa* were mainly resistant to cephalosporin's and carbenicillin; For *E. coli* were broad-spectrum beta-lactamase-producing strains (ESBL) resistant to cephalosporin's, trimethoprim and sulfamethoxazole, ciprofloxacin and norfloxacin; in the case of *K. pneumoniae* (ESBL) were resistant to cephalosporin's, aminoglycosides, trimethoprim and sulfamethoxazole, tetracycline, ciprofloxacin and norfloxacin.

**Bactericidal effect of the ethanolic extracts of the different medicinal plants:** The results obtained showed that ethanolic extracts had a bactericidal activity against all strains assayed in the study. Importantly, the MICs presented in Table 1, represent the average of the concentrations of the extract evaluated by each species. So, the extract of mangle red against wild strains of *Pseudomonas aeruginosa* demonstrated a good bactericidal effect because it showed a lowest MIC´s with a concentration at 1.25 mg/mL, followed by the extract of lemon peel of 4.0 mg/mL.

**Table 1. Average of mic's of extracts tested**

| BACTERIA | ONION | GARLIC | LEMON | MANGLE |
|---|---|---|---|---|
| *P. aeruginosa* | 6.0 | 6.0 | 4.0 | 1.25 |
| *E. coli* | 8.0 | 8.0 | 8.0 | 2.50 |
| *K. pneumoniae* | 8.0 | 8.0 | 8.0 | 2.50 |

For the strains of *Escherichia coli* and *Klebsiella pneumoniae*, the MIC's reached a concentration equivalent to twice that concentration used for *P. aeruginosa*. Therefore, the ethanolic extract with the best bactericidal activity for bacteria assayed was the red mangrove bark, with a MIC whose extract

concentration was up to three times lower than that used for other extracts.

## DISCUSSION

This work showed that the ethanol extracts of the bark of *R. mangle* had the best antimicrobial activity, with an effect up to three times more potent against bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*, and up to almost five times in relation to *P. aeruginosa*, in comparison with the others extracts. This coincides with a report where they found bactericidal activity in extracts of this mangle against bacteria of interest in humans, although they did not report the minimum inhibitory concentration (Regalado, 2016). Likewise, our ethanolic garlic and onion extracts demonstrated an antimicrobial effect against the bacteria tested, observing the same MICs in both extracts. These results are similar to a report in which alcohol extracts of garlic killed strains of *P. aeruginosa*, althought had not activity against *E. coli* (Chalar, 2014). Regarding the onion, a work done with the alcoholic extracts of this vegetable had no bactericidal effect, differing from what was reported in our work (Dávila, 2013). Finally, as stated in this report, our ethanolic extracts of the lemon peel presented antimicrobial activity against all species tested in the study, being in agreement with another work where they found the same antimicrobial activity when using the essential oils of this citric (Argote, 2016).

### Conclusion

The ethanolic extracts of the red mangle bark evaluated demonstrated a bactericidal activity against all the multi-resistant gram-negative bacteria tested.

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