

Polyextracts of medicinal plants vs bacteria associated with acute respiratory infections (IRAS)

Rosa Iris Mayo Tadeo¹
Mónica Espinoza Rojo¹
Javier Jiménez Hernández¹
Flaviano Godínez Jaimes²
Agustín Damián Nava³
Dolores Vargas Álvarez^{1§}

¹Autonomous University of Guerrero-Faculty of Chemical Biological and Biomedical Sciences-CU. Avenue Lázaro Cárdenas s/n, col. The haciendita, Chilpancingo de los Bravo, Mexico. CP. 39090. (roxina071.iris@hotmail.com; monespinoza@yahoo.com; jjimenezuagro@gmail.com). ²Autonomous University of Guerrero-Faculty of Mathematics. Avenue Lázaro Cárdenas s/n, col. The haciendita, Chilpancingo de los Bravo, Mexico. CP. 39090. (fgodinezj@gmail.com). ³Autonomous University of Guerrero-Faculty of Agricultural and Environmental Sciences. Iguala de la Independencia, Guerrero, Mexico. (agudana@yahoo.com.mx).

§Corresponding author: dvargas@uagro.mx.

Abstract

The objective of this research work was to model the preparation of a syrup formulation based on plant extracts of bougainvillea, eucalyptus, cinnamon, marigold and itamo to inhibit the growth of *S. aureus* and *S. pyogenes*, bacteria associated with respiratory infections. The presence of secondary metabolites in the methanolic extracts of each species was determined by chromatography, as well as the antibacterial activity of the extracts and syrups and their antioxidant capacity were evaluated. Eucalyptus extract was found, to be the best at inhibiting bacteria, just like its syrup form, and cinnamon extract is the best antioxidant.

Keywords: antibacterial activity, antioxidant activity, plant extracts, secondary metabolites.

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Since ancient times, different health preservation methods have been used through herbalism. Currently, France is the country that has most rebounded in obtaining new strategies for generating knowledge about natural products, for each process of elucidation of metabolites to identify the active ingredients (Harbone, 1997).

However, since time immemorial the indigenous Mexican cultures have used to collect the hazards every year on the first Friday of March in the translation from winter to spring, preamble to the flowering of March 21 when spring frenzy enters. It is customary to collect flowers, flower buds and bark that are poured into massage oil or liquor for massages and drinking, which indicates that it is a sustainable and drinkable solvent suitable for human consumption.

In the Popoluccan, Náhuatl, Mixtec and Totonac cultures this mixture is called yolispa, cured, heal everything, digestive, relaxing and it is customary to store in a dark place and in earth pits, in order to preserve it until it is occupied (Argueta *et al.*, 2000). In the construction of scientific thought, they are polyextracts, which have innumerable functions because they go beyond the objectives of many antibiotics from antimicrobial and antioxidant activity (Márquez *et al.*, 2005).

The imbalance that causes oxidative stress and the balance of the balance of the flora through the conjunction of its extracted metabolites in a moderately polar solvent, this comparative advantage generates the arrest of oxidative stress, maintaining stable human health and even of any living being, for therefore, this work aims to reveal the behavior of the extracts individually and combined in the antimicrobial and antioxidant activity.

The plant material consists of five species of plants, from which red and purple bougainvillea flowers, eucalyptus fruits, cinnamon bark and leaves of itamo obtained from different places were obtained. The samples were collected in the Acahizotla community, municipality of Chilpancingo, Guerrero. Two varieties of marigold (S2 and S3) were studied. For cinnamon and eucalyptus the plants were separated into parts, washed with water and dried at room temperature.

For bougainvillea and the itamo, they were placed in perforated paper bags in a convection stove at a temperature of 60 °C, turning them daily for three days. To obtain the extracts, 60 g of each sample were weighed adding 500 ml of 95% ethanol, using the soxhlet method to different washing cycles. Once the extracts were obtained they were concentrated on a rotary evaporator until obtaining a minimum volume, to then allow them to dry at room temperature in order to evaporate the rest of the solvent and thus obtain a crude extract, and a paper chromatography was performed to verify the bands. of the metabolites present as a quality control.

Bacterial strains of *S. pyogenes* and *S. aureus* were used. Culture media Mueller-Hinton agar for *S. aureus* and blood agar for *S. pyogenes* were selected. The inoculum of the bacterial strains to be used was first prepared, adjusting them with a 0.5 nephelometer on the MacFarland scale, corresponding to a concentration of 1.5×10^8 cells ml^{-1} . The evaluation of antibacterial activity was carried out with the Kirby-Bauer method. The positive control was a 10 µg penicillin disc and the negative was a disc with 20 µl ethanol. Each of the tests was performed in triplicate. On the other hand, the antioxidant activity of the extracts of each plant was determined, it was carried out by the DPPH method (1,1-diphenyl-2-picrilhydrazyl).

The results were expressed as IC₅₀ (in mg ml⁻¹) inhibitory concentration, that is, the amount of substance in 1 ml of reaction, necessary to decrease the initial concentration of DPPH to 50%. To make a syrup, all components of the formula were weighed, honey was added to the water and gently stirred until a homogeneous mixture was obtained, then a dye (microencapsulated from Jamaica variety Tecuanapa) was added as a product of a result of another investigation): immediately the flavoring was incorporated, finally, the active ingredient was added and the mixture was gently stirred. To evaluate the inhibitory effect of the formulations obtained on the growth of bacterial strains, the same methodology was used for the crude extracts of the five plants, placing 10 µl on each disk.

Treatment averages for calendula-based formulas have very small inhibitory halo means, by contrast, some penicillin-based formulas have the best results, this can be seen in Figure 1.

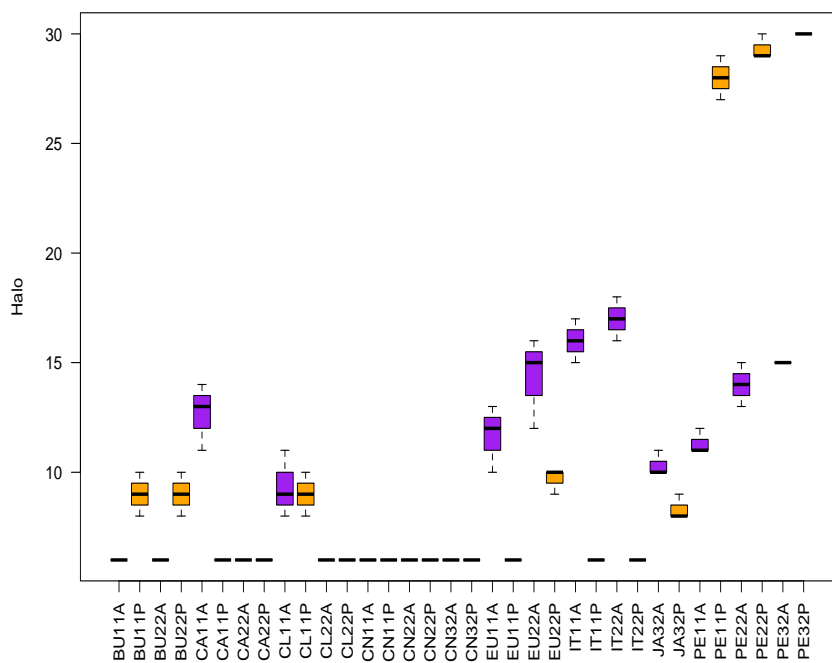


Figure 1. Distribution of the inhibition halos (cm) of the statistical treatments studied.

In the previous figure, the results obtained from the statistical analysis of the antibiograms performed on the samples used are described. With the positive controls, in this case, a disk with 10 µg of penicillin was used, and the negative control was a paper disk with 20 µl of alcohol with a diameter of 6 mm. (BU= bougainvillea, calendula, CA= cinnamon, negative control, eucalyptus, itamo, syrup and penicillin.

For the *S. aureus* strain, the BU11A treatment presented a range of 24.5, as did the CN11A negative control, while the CL11A treatment obtained the lowest inhibition range of 60, that is, the inhibitory power of this treatment is minimal. It was also observed that against this microorganism the treatments that presented a higher range were: IT11A, followed by CA11A and EU11A, which had values of 88.33, 76.5 and 72.5 above PE11A, presenting a range of 72.33 that was used as a positive control.

In Figure 1 for *S. pyogenes*, it is shown that the CA22P, CL22P and IT22P treatments did not show inhibition halos having a mean of range equal to CN22P (negative control), whereas BU22P syrups have a mean of range of inhibition of 57.83 and the EU22P presented a mean of range of 62.17, PE22P (antibiotic) was the one that obtained a greater halo of inhibition this because it is a specific treatment for this bacterium.

In the *S. aureus* strain, it is observed that the syrups BU22A, CA22A, CL22A have inhibition rank means of 24.5 as well as CN22A (negative control), on the other hand, EU22A treatments have a value of 82.83 e IT22A of 91.17 of inhibition range means greater than PE22A having a range of only 81 (antibiotic) which was used as a positive control.

The sample of the PE32P treatment (positive control), since this is a specific antibiotic for these bacteria, while the syrup (JA32A) presented higher sensitivity against *S. aureus* with a range of 66.67, compared to *S. pyogenes* with a value of 53.5. Figure 1 shows that all plants had antioxidant activity to different degrees, with cinnamon having the highest antioxidant activity, which was reflected in the statistical analysis of the IC50 (Amount of extract needed to reduce 50% of the radical), in which the best antioxidant is the one that requires the least amount to reduce only 50% of the free radical (DPPH), as shown in Table 1.

Table 1. Antioxidant activity of methanolic extracts from plants.

Species	Antioxidant potential (%)	CI50 (mg) [§]
Cinnamon	100 ±0	0.25 a
Eucalyptus	29.9 ±0.3	0.8 c
Calendula (S2)	33.1 ±0.3	0.7 c
Itamo	12.1 ±0.8	2.08 d
Red bougainvillea	73.7 ±3.5	0.34 b

±= standard deviation. §= means with different letters are statistically different ($\alpha=0.05$).

Based on the results obtained from the antibacterial activity of the extracts against *Staphylococcus aureus*, it is established that the extracts of cinnamon, itamo, calendula and eucalyptus (García *et al.*, 2004) function as bacterial inhibitors against these strains using 20 μ l (Figure 1). Studies carried out by Márquez *et al.* (2005) demonstrate that the itamo can inhibit *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomona aeruginosa* with 10 mg ml⁻¹ of ethanolic extract.

Lu *et al.* (2011) states that cinnamon essential oil is a strong antibacterial agent against *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli* and *S. typhimurium*. Martin *et al.* (2010) in the investigation of chemical composition and antimicrobial properties of the essential oil of three eucalyptus species demonstrate the antibacterial activity against *S. aureus*. Lastra and Piquet (1999) showed the antibacterial activity of calendula especially against *S. aureus*, *S. fecalis*, *S. Schipochliev* and *S. Fleischner*.

The extract of bougainvillea was the only one that did not inhibit the bacteria, as established in a study by Cáceres (1990); Abarca-Vargas and Petricevich (2018), in which he demonstrates that the aqueous and ethanolic extracts of bougainvillea leaves and flowers are inactive against *E. coli* and *S. aureus*. However, it is desirable to use higher concentrations of the extract to corroborate inactivity. *Streptococcus pyogenes* was sensitive to eucalyptus and bougainvillea extracts (Figure 1), presenting resistance to the other extracts, this may be due to the fact that the bacterium has a capsule and M protein, which are associated with greater virulence (Alos *et al.*, 2003).

Avila *et al.* (2006) related the antibacterial action against *S. aureus* to the presence of terpenes and flavonoids in the *Diplostephium tominse* species. Tabares *et al.* (2007) described that the identification of triterpenes in a plate reveal the connection between their chemical composition, since they cross the surface barrier of bacteria and then attach to their cell membrane.

The activity of flavonoids against microorganisms is probably due to the fact that they form complexes with soluble, extracellular proteins and with cells of the bacterial wall. The antimicrobial activity of tannins is believed to be due to their interaction on adhesins, cell wall proteins, and their ability to bind to polysaccharides (Cowan, 1999).

It is worth mentioning that the formulations carried out have a varied pH ranging from 5.1 to 5.5 and brix degrees of 58.9, values that are within the limits established by NMX-F-169-1984. PH is one of the quality factors to control in syrups, as well as indicators of hygienic conditions; a low value of this favors the inactivation of microorganisms (FAO, 2004).

In the evaluation of the antioxidant activity carried out by the DPPH method, we observed that the cinnamon extract showed the highest antioxidant capacity in 100% at a concentration of 0.1 g ml⁻¹ of methanolic extract, likewise Castañeda *et al.* (2008) demonstrated the antioxidant capacity of the cinnamon extract obtaining 90.11% of the antioxidant potential at a concentration of 1 ug ml⁻¹. Extracts of bougainvillea, calendula, eucalyptus (García *et al.*, 2004) and itamo that had a lower antioxidant potential compared to cinnamon, which is reflected in Table 1.

The organism is exposed to a great variety of ROS (reactive oxygen species) and RNS (reactive nitrogen species) that can be generated from endogenous sources, related to oxygen metabolism and the various defense reactions of our immune system (Dreosti, 2000; Osorio *et al.*, 2009).

In respiratory infections, there is a large increase in phagocytic cells (neutrophilic leukocytes, macrophages, and eosinophils), which, when activated by proinflammatory mediators or by bacterial, viral, or parasitic products, destroy infected cells by means of an oxidative attack in which large quantities of O₂⁻, H₂O₂, OH, NO and OCl⁻ are produced (Bravo and López Ortega, 1998). It is important to evaluate the antioxidant activity of the extracts to help retard or inhibit oxidative stress in the treatment of many diseases and infections.

Conclusions

Cinnamon showed a greater antioxidant capacity compared to the other extracts used, the mixture used with the five extracts worked better as an inhibitor against the *S. aureus* strain, eucalyptus presented the highest antibacterial inhibition halo as extract alone, as well as in syrup form against *S. aureus* and *S. pyogenes*.

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