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Aloe Vera L and Croton Lechleri Resins as Antibacterial Potential in Drinking Water in the Peruvian Jungle

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The present investigation was carried out to evaluate the antibacterial effect of *Aloe vera* and *Croton lechleri* resin in water for human consumption. The study method was quasi-experimental with pre-test and post-test. *Aloe vera* resin and *Croton lechleri* at concentrations of 25%, 50% and 75% volume were used as biological samples, each concentration corresponding to a treatment, i.e., T1 as control with calcium hypochlorite at 0.5 ppm, T2, T3 and T4. It was determined that there is water consumption with high levels of total coliforms (925 CFU/100mL), thermotolerant coliforms (845 CFU/100mL) and *Escherichia coli* (1110 CFU/100mL), whose values exceed the maximum permissible limits according to Peruvian Supreme Decree regulations. The research concludes that the treatment with a higher concentration of aloe resin is able to inhibit the concentration of microbial load to a greater extent. Thus, Treatment (T4) achieved a greater bactericidal effect of 100% on total Coliforms, thermotolerant Coliforms and *Escherichia col*i, followed by Sangre de grado (*Croton lechleri*) resin, decreasing by 99%. It is important to mention that plants and their derivatives are an efficient and effective alternative for the elimination of bacteria present in water samples, being an ecological proposal for the care of natural resources and human health.

1. Introduction

Water represents an important element that is impossible to replace, but it is a cause for concern due to its limitations as processes and sanitary measures for its disinfection and decontamination in accordance with public health regulations (Dariva and Araujo, 2021; Grunwald, 2016). Half of the population lacks adequate water and sanitation due to the increased demand for resources in urban, agricultural, commercial, industrial and mining areas (Pereira et al., 2014; Sen, 2015). However, the design of measures to recover, sanitize and reuse water through less expensive, more efficient and easier to apply techniques is being promoted (Jasim, 2020). The absence of adequate means of sanitary treatment turns out to be an infectious focus causing serious digestive diseases transmitted by the consumption of water contaminated by microorganisms such as total coliforms, thermotolerants and *Echerichia coli* (Grasso, 2019); 4% of all deaths worldwide are linked to problems related to water, sewage and hygiene (Rodriguez et al., 2016).
In 2015, the lack of this vital liquid affected approximately 663 million people; in view of the problem, the Millennium Development Goals (MDGs) were defined with normative rights for access to drinking water and sanitation in accordance with national policies (Giné et al., 2017; Who/Unicef, 2015). Based on these objectives, a system for improving quality of life and sustainable development is provided for the 2030 Agenda (Jasim, 2020; Queiroz et al., 2020). Water pollution is caused by the use of chemical substances, lack of segregation of solid waste, indiscriminate use of fertilizers, among others, which are discharged into the soil, rivers and seas, causing a public health problem and damaging the environment (Latorre and Tovar, 2017; Canaza, 2018).

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The rapid growth of the population generates a greater demand and at the same time the need to use sustainable methods that promote new technologies for the treatment of water for human consumption. To counteract this, technologies have been created that use plants as a primary source, calling them biological treatments or bioremediation processes using extracts, leaves, seeds, fruits, gels or resins to remedy resources such as soil, water or air (Caviedes et al., 2016, Chen et al., 2016; Sun et al., 2016). In this sense, there is a growing need to innovate in methods that improve water quality and, simultaneously, eliminate microorganisms that cause a health problem in society. The use of plant resins is a very suitable technique to reduce pollutants in water and improve its quality, since some plants can easily accumulate pollutants through different physical and biochemical mechanisms (Manoj et al., 2020). In fact, phytoremediation can be considered more economical compared to other techniques such as adsorption and filtration (Wan et al., 2016; Willscher et al., 2017).

2. Method

2.1. Study area

The study was developed in coordination with the authorities of the administrative board of local sanitation services located in the township of La Planicie, Morales district. Geographically, the inhabitants' water supply is located in the district of Cacatachi, department of San Martin with its geographical coordinates: East: 345533 and North: 9284965 in zone 18 South WGS 84, at an altitude of 280 meters above sea level.

2.2. Sampling of water for human consumption

Water samples were taken according to the Protocol of procedures for sampling, preservation, conservation, transport, storage and reception of water samples for human consumption (DIGESA, 2015). Four water samples of 500 ml each were collected and stored in sterile glass bottles, taking into account the protective barriers (sterile gloves, apron and boots) to avoid being contaminated or contaminating the sample. The samples taken were kept refrigerated at an average temperature of 4 to 8°C, in a cooler, until they were transported and processed at the laboratory of the Inspection & Testing Services of Peru, where they were analyzed for total coliforms, thermotolerant coliforms and Escherichia coli (D.S. N°.031 - 2010 - S.A. of the Ministry of Health).

2.3. Identification of microorganisms in drinking water samples

Microorganisms of the total coliform group were identified, for which 15 screw-capped test tubes (3 rows each with 5 tubes) containing 10 mL of lauryl sulfate broth (Merck) and Durham's hood were prepared; 10 mL of water for human consumption was poured to 5 tubes of the first row, 1 mL of water to 5 tubes of the second row, 0. 1 mL of water to the 5 tubes of the third row, and allowed to incubate for 24 hours at 37° C in the oven (Memmert GmbH + Co. KG). The reading was made considering positive those tubes that presented turbidity and gas (elevation of the Durham hood), these positive tubes were separated. For the confirmatory test, the tubes separated as positive were seeded in screw cap tubes and mixed uniformly with the Digralsky loop containing 10 mL of brilla broth (Merck) with the Durham hood. These tubes were incubated at 37°C for 24 hours, positive tubes (presence of gas and turbidity), were counted and compared to the most probable number multiple tube table. Finally, for the identification of Thermotolerant Coliforms and *Escherichia coli*, the positive tubes confirmed through the brilla Broth were separated, after which 10 mL of the sample was obtained and seeded in tubes with screw cap placing the Durham hood and leaving it to incubate at 44.5°C for 24 hours in a water bath. To the positive tubes (turbidity and gas) 3 drops of Kovacs reagent were added and 2 minutes were waited to visualize the formation of a cherry-colored ring on the surface of the broth; the presence of the cherry ring indicates positive (DIGESA, 2015).

2.4. Phytochemical analysis of Aloe vera and *Croton lechleri* resins

The resin of the plant species was evaluated in order to identify their active principles. Each sample was subjected to solvents of increasing polarity, to obtain secondary metabolites according to their solubility, using reagents and dyes to determine the presence or absence of active components such as: steroids, triterpenes, quinones, phenolic compounds, flavonoids, lactones, alkaloids, reducing sugars, tannins and saponins, using the protocol described by Lock (2016). Two mL of pure extract was added to 10 test tubes to identify secondary metabolites through color change, classified as light, moderate or strong. The tests used to determine the presence of each type of secondary metabolite are listed in Table 1.

2.5. Preparation of inhibitory solution

The resin was obtained by applying a diagonal cut to the stem of the *Croton lechleri* and *Aloe vera* species. From the leaf skin, the cut area was previously disinfected with alcohol, the resin was received in a sterile flask, and dilutions were made according to the solute volume/solution volume ratio, For the concentrations, sterile

distilled water was used in order not to alter the physicochemical characteristics of the resins; a control group of 1 ppm calcium hypochlorite (12 mg + 1 liter of sterile distilled water) and 3 treatments were used: treatment 1; 25% (25 mL of resin + 75 mL of sterile distilled water). Treatment 2: 50% (50 mL resin + 50 mL sterile distilled water); treatment 3: 75% (75 mL resin + 25 mL sterile distilled water); concentrations were kept in sterile bottles and refrigerated. (Tamariz et al, 2003; Salamanca, 2014).

Table 1. Phytochemical analysis of medicinal plant resins from the Peruvian Jungle.

Test	Secondary metabolites	*Aloe vera*	*Croton lechleri*
Lieberman-Bouchard	Steroids and triterpenes	(+++)	(++)
Borntrager	Quinones	(+)	(-)
Ferric chloride	Phenolic compounds	(+++)	(++)
Shinoda	Flavonoids	(++)	(++)
Baljet	Lactones	(+)	(-)
Dragendorff	Alkaloids	(+)	(++)
Mayer	Alkaloids	(+)	(+)
Fehling	Sugar reducers	(++)	(+)
Gelatin	Tannins	(++)	(+)
Foam	Saponins	(+)	(-)

Note: Color changes of secondary metabolites in (+) = slight, (++) = moderate and (+++) = strong

2.6. Inhibition experiment protocol

Mueller-Hinton agar culture medium (Merck) was prepared under biosafety conditions; 8 mL was poured into 20 100 mm Petri dishes (2 plates for the control group and 3 plates per treatment for each species) and allowed to dry at 37 °C for 30 minutes. Subsequently, 0.5 mL of the total coliform, thermotolerant coliform and *Escherichia coli* positive tubes were added to the agar plates and incubated at 37°C for 24 hours to observe colony growth. Then 0.5 mL of calcium hypochlorite 1 ppm was added (2 plates as control group), 0.5 mL of treatment 1 (6 plates: 3 with *Aloe vera* resin and 3 with *Croton lechleri*), 0.5 mL of treatment 2 (6 plates: 3 with *Aloe vera* resin and 3 with *Croton lechleri*), 0.5 mL of treatment 3 (6 plates: 3 with *Aloe vera* resin and 3 with *Croton lechleri*), 0. 5 mL of treatment 3 (6 plates: 3 with *Aloe vera* resin and 3 with *Croton lechleri* plates) were incubated at 37°C for 24 hours, after 24 hours the number of colonies was counted.

2.7. Determination of the inhibition efficiency of *Aloe vera* and *Croton lechleri* resins

The percentage difference formula was used to determine the percentage of efficiency, taking into account the control group and the experimental groups, as follows:

$$\text{Inhibition efficiency (\%)} = \left[\frac{CGC - CGE}{CGC}\right] \times 100 \quad (1)$$

Where:
CGC: Control group concentration
CGE: Experimental group concentration

3. Results

The treatments were applied using aloe vera resin. For total coliforms, the control group had 420 UFC/100 ml of water, treatment 1 had 210 UFC/100 ml of water, treatment 2 had 65 UFC/100 ml of water and treatment 3 had 0 UFC/100 ml of water. For thermotolerant coliforms, the control group had 390 UFC/100 ml of water, treatment 1 had 257 UFC/100 ml of water, treatment 2 had 82 UFC/100 ml of water and treatment 3 had 0 UFC/100 ml of water. For *Escherichia coli*, the control group had 690 UFC/100 ml of water, treatment 1 had 320 UFC/100 ml of water, treatment 2 had 92 UFC/100 ml of water and treatment 3 had 0 UFC/100 ml of water. The results show that the control group decreased the number of UFC/100 ml in all parameters compared to the analysis of a water sample without treatment. However, the resin treatments have decreased the number of microorganisms per parameter, so the higher the concentration, the greater the inhibition of bacteria.

Table 2: Parameters of untreated water for human consumption

Parameters	Unit of measure	Untreated water	Regulations LMP
Total coliforms	UFC/100 mL a 35ºC	925	0
Thermotolerant coliforms	UFC/100 mL a 44.5°C	845	0
Escherichia coli	UFC/100 mL a 44.5°C	1100	0

The analysis of a sample of untreated water showed 925 UFC/100 mL of total coliforms, 845 UFC/100 mL of thermotolerant coliforms and 1100 UFC/100 mL of *Escherichia coli*, which exceeded the unit of measurement and did not comply with the Peruvian standard of maximum permissible limits (DIGESA, 2010).

Table 3: Control group and treatments with Aloe vera resin on microorganisms.

Parameters	Unit of measure	Control group	Treatment in doses of *Aloe vera* resin		
		Calcium Hypochlorite	T1 25%	T2 50%	T3 75%
Total coliforms	UFC/100 mL a 35º C	420	210	65	0
Thermotolerant coliforms	UFC/100 mL a 44.5° C	390	257	82	0
Escherichia coli	UFC/100 mL a 44.5° C	690	320	92	0

Table 4: Control group and resin concentrations of Croton lechleri in microorganisms.

Parameters	Unit of measure	Control group	Treatment in doses of *Croton lechleri*		
		Calcium Hypochlorite	T1 25%	T2 50%	T3 75%
Total coliforms	UFC/100 mL a 35º C	420	264	58	3
Thermotolerant coliforms	UFC/100 mL a 44.5° C	390	218	61	7
Escherichia coli	UFC/100 mL a 44.5° C	690	297	72	4

The treatments were applied using *Croton lechleri* resin, showing that in total coliforms the control group had 420 UFC/100 mL of water, treatment 1 had 264 UFC/100 mL of water, treatment 2 had 58 UFC/100 mL of water and treatment 3 had 3 UFC/100 mL of water. For thermotolerant coliforms, the control group had 390 UFC/100 mL of water, treatment 1 had 218 UFC/100 mL of water, treatment 2 had 61 UFC/100 mL of water and treatment 3 had 7 UFC/100 mL of water. For *Escherichia coli*, the control group had 690 UFC/100 mL of water, treatment 1 had 297 UFC/100 mL of water, treatment 2 had 72 UFC/100 mL of water and treatment 3 had 4 UFC/100 mL of water. The results show that the growth of bacteria has not been completely inhibited according to the parameters. However, a higher concentration could be considered to achieve total inhibition and meet the quality standards according to Peruvian regulations

Table 5: Percentage efficiency of treatments on Aloe vera resin

Parameters	Control group (% efficiency)	% of efficiency between treatments		
		T1 25%	T2 50%	T3 75%
Total coliforms	45	50	84	100
Thermotolerant coliforms	46	34	78	100
Escherichia coli	62	53	86	100

The results in the table show the percentage of efficiency of the *Aloe vera* resin treatments compared to the control group. In the total coliform parameter, the control group had an efficiency of 45% compared to treatment 1 which had 50%, treatment 2 had 84% and treatment 3, 100%. For thermotolerant coliforms, the control group had 46% efficiency compared to treatment 1 which had 34%, treatment 2 had 78% and treatment 3 had 100%.

Finally, for the *Escherichia coli* parameter, the control group had 62% efficiency compared to treatment 1 which had 53%, treatment 2 had 86% and treatment 3 had a value of 100%. It is evident that treatment 3 with 75% concentration is more efficient and eliminates all microorganisms, so it could be a phytoremediation alternative to maintain microbiological properties within the indicators of water quality for human consumption.

Table 6: Percentage efficiency of treatments on resin of Croton lechleri

Parameters	Control group (% efficiency)	% of efficiency between treatments		
		T1 25%	T2 50%	T3 75%
Total coliforms	45	37	86	99
Thermotolerant coliforms	46	44	84	98
Escherichia coli	62	56	89	99

The results in the table show the percentage of efficiency of the *Croton lechleri* resin treatments compared to the control group. In the total coliform parameter, the control group had an efficiency of 45% compared to treatment 1 which had 37%, treatment 2 had 86% and treatment 3, 99%. For thermotolerant coliforms, the control group had 46% efficiency compared to treatment 1 which had 44%, treatment 2 had 84% and treatment 3 had 98%. Finally, for the *Escherichia coli* parameter, the control group had 62% efficiency compared to treatment 1 which had 56%, treatment 2 had 89% and treatment 3 had a value of 99%.

4. Conclusions

The analysis of the water sample for human consumption did not comply with Peruvian regulations, despite the existence of a Supreme Decree for the maximum permissible conditions or limits for drinking water consumption. This situation has probably generated health problems in the inhabitants. In response to this situation, medicinal plants were used, such as *Aloe vera* resin and *Croton lechleri*, due to their great phytochemical properties. In order to inhibit microbial growth in drinking water different concentrations were used. In addition, a control group (calcium hypochlorite) was used for comparisons, so that *Aloe vera* treatment 4 (75% concentration) has a higher removal of microorganisms. No UFC/100 mL were found for total coliforms, thermotolerant coliforms and *Escherichia coli*, followed by Croton lechleri resin where 3 UFC/100 mL were found for total coliforms, 7 UFC/100 mL for thermotolerant coliforms and 4 UFC/100 mL for *Escherichia coli*. The percentage of efficiency was evaluated and found to be 100% in treatment 4 of *Aloe vera* and 99% in *Croton lechleri*. It is important to mention that the use of medicinal plants could be an efficient, effective and less costly alternative that would help minimize the negative impacts or contamination of the water resource.

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