Illustration: Mauricio Vázquez Rendón.

Behind Natural Negative Refraction

Juan Diego Mazo Vásquez

Physicist. Student of the Master's Degree in Optics and Photonics. Theoretical Group of Materials Sciences. Faculty of Exact and Natural Sciences, Universidad de Antioquia. Universidad de Antioquia Student Research Award 2020, first category.

Mathematical models allow us to describe what happens on very small scales. In this research, they were used to explore how light would behave when interacting with a gas in which electrons are free and to answer whether this interaction could naturally cause negative refraction.

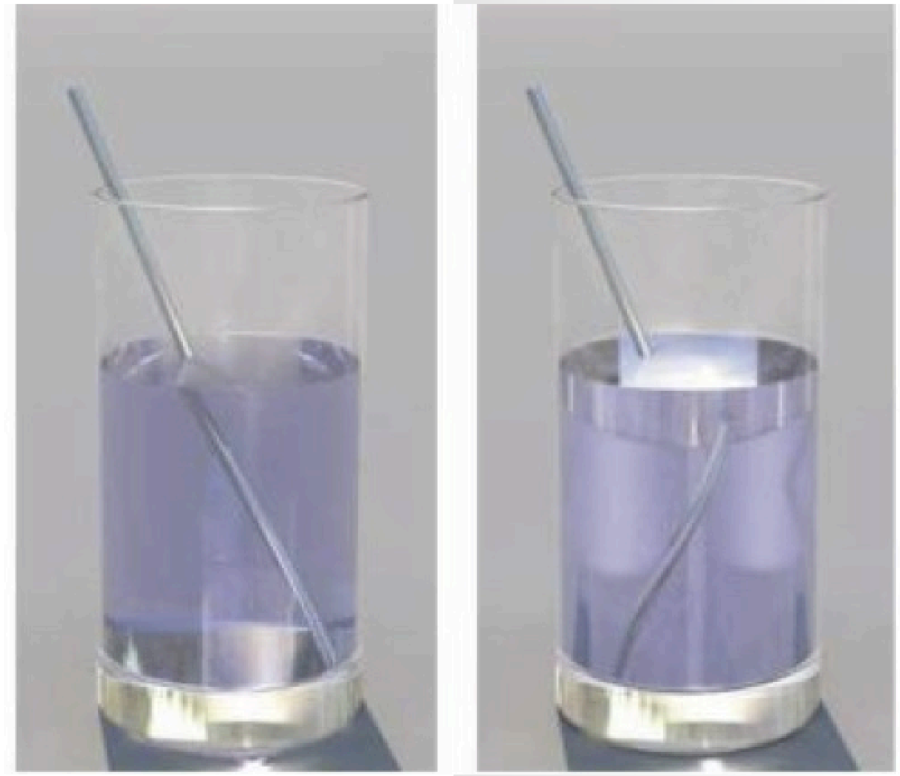
It's a hot day. The bright sunshine makes the weather perfect for a dip in the pool. Suddenly, we notice a coin at the bottom of the pool. We try to locate it and dive in to pick it up. The moment we dive in, we realize that it is not exactly where we thought it was.

The explanation for this phenomenon is that the light that reaches our eyes does not follow a straight path but is deflected when the medium in which it propagates changes, for example, when it passes from water to air. This phenomenon is known as **refraction.**

The deflection of light in a different medium is, in a way, something close to our senses because we can witness it every day in various ways, such as when we put a pencil in a glass of water and see it "break". Light does not follow the trajectory that minimizes the distance, but the one that makes the travel time minimum. And although physicists have managed to understand the mechanism of refraction, we are still not entirely clear about many aspects related to another case: when light is deflected in the opposite direction. That is, the phenomenon of positive refraction occurs when light is refracted in the same direction in which it arrives, while negative refraction is when light is deflected in the opposite direction.

The idea of negative refraction was formulated by the Russian physicist Viktor Veselago, who in 1968 put forward the possibility of substances that could deflect light in the opposite direction to the original one. This opened the door to research into metamaterials both theoretically and experimentally because negative refraction leads to many other interesting optical phenomena and can be used to design devices such as antennas and lenses.

In the 1990s, the American physicist John Pendry proposed the construction of the first metamaterial: a structure made of metallic rings that, under certain conditions, could recreate the phenomenon of negative refraction. In subsequent years, many modifications have been made to Pendry's model to develop antennas with better reception and transmission quality for signals such as radio or visible light. In addition, the possibility of implementing panels with metamaterials has been investigated to increase, for example, the processing capacity of technological devices such as computer processors and military applications to improve camouflage techniques.



On the left is an example of positive refraction. On the right is a representation of what the refraction of light would look like if water were a metamaterial, i.e., an example of negative refraction. Image: Phys.org

Although it has been possible to design artificial metamaterials, we do not know of any natural one. For this reason, Universidad de Antioquia's Theoretical Group of Materials Sciences studied whether light could manifest negative refraction in a gas of electrons, called relativistic Fermi gas, considering that in recent years it has been postulated as a good candidate.

A relativistic Fermi gas is composed of electrons, which are considered non-interacting. This type of gas is found at high densities and temperatures, and is present, for example, in the plasma present in stars. When this gas interacts with light, electrons begin to vibrate and scatter the light in different directions. Under certain conditions, it may manifest negative refraction.

To understand how light is scattered by the presence of the gas, the equations that govern electromagnetic fields and special relativity are used. In addition, the behavior of the refractive index is studied. The refractive index is a quantity that determines the propagation of light within a medium depending on several factors, such as temperature, particle density and incident radiation frequency. In the case of the materials we know, we find that the refractive index is a positive number, but in the case of metamaterials, it is expected to be negative so that negative refraction can occur.

In the case of electron gas, we find that the refractive index is a complex number, that is, it is neither a positive nor a negative number, and therefore, it cannot be concluded that negative refraction occurs. Despite the above, the fact that it is a complex number accounts for the amount of energy that light delivers to the particles and that is used in the creation of antiparticles in the gas together with the energy they acquire because of the temperature.

On the other hand, one of the most important results of the group's research is that a certain portion of the light that interacts with the electron gas does not deviate from its original direction, i.e., it propagates as if no particles were present.

In addition, it was found that, if the gas is under certain temperature conditions and the light incident on it has certain frequencies, the light wave may propagate in the opposite direction to the energy in the wave, which is one of the implications of Veselago's ideas. However, to have more precise conclusions, it is necessary to carry out experimental research to verify the theoretical results we obtained.

The results, although preliminary because of the lack of experimental corroboration, can also give us ideas about what happens in the interstellar medium and the stars. Moreover, since it is a relatively simple model with several considerations, the underlying physics is found to be valuable for the understanding of many phenomena and could be useful for industrial development. **X**

Experimentally verifying these results is very difficult because it would require recreating a system that is under the same conditions as the material of the stars and having the ability to measure how light behaves in the presence of the gas under very specific conditions.

Glossary

Antenna: part of a radio or television set from which waves are transmitted into the atmosphere or space.
Metamaterial: a type of material with properties that do not usually occur naturally, such as a negative refractive index.
Complex number: a number that has a real and an imaginary part.
Refraction: change of direction undergone by a wave when passing from one medium to another.

Culture of fibroblasts necessary for the construction of the skin equivalent.
Photo: Communications Office.

Catalina Gaviria Agudelo

Bioengineer, MSc in biomedical engineering, PhD candidate in basic biomedical sciences. Member of Universidad de Antioquia's Tissue Engineering and Cellular Therapies Group (GITTC). Member of the University of Groningen's Laboratory of Genomic Instability in Development and Disease Group.

Floris Foijer

Biotechnologist, PhD in molecular biology. Coordinator of the Laboratory of Genomic Instability in Development and Disease Group.

Luz Marina Restrepo Múnera

Biologist, PhD in sciences. Professor of the Faculty of Medicine and coordinator of Universidad de Antioquia's Tissue Engineering and Cellular Therapies Group (GITTC).

Skin substitutes are reconstructions made from human cells. They can help study human skin and test potentially harmful substances, which makes them a powerful alternative to the use of laboratory animals.

Creating Skin in a Laboratory

Incredible as it may seem, every hour we lose more than one million dead cells from the surface of our skin. Therefore, new cells must constantly be born to replace those lost and form the outermost layer of the skin: the epidermis. This is the visible part of the skin and is mostly composed of cells called keratinocytes. These are constantly dividing and changing function and shape as they make their way to the surface of the skin. There they stay briefly and then begin to die and slough off, which causes the whole cycle to repeat itself.

We find the innermost layer of the skin known as the dermis beneath the epidermis. It is composed mainly of cells called fibroblasts, which are responsible for producing proteins and other components that form the network where the cells reside, i.e. the extracellular matrix (ECM).

These two layers work together to cover the entire body and protect us against harmful external agents. Interestingly, it was thought until recently that the skin had no immune role, and it was only an envelope that protected the body's organs. However, it is now known that the skin actively participates in the immune system thanks to the presence of so-called dendritic cells. These interact with foreign agents and can trigger an immune response.

Production of Skin Substitutes

At GITTC, we conducted a study in which we sought to create skin substitutes with an immune component to evaluate the sensitizing potential of chemical substances. The first step was to obtain the cells. We started with blood and skin fragments obtained from biopsies or reduction surgeries. Thanks to enzyme treatments, which break the bonds between cells, and between cells and the ECM, we were able to obtain keratinocytes and fibroblasts from the biopsies. On the other hand, since blood contains many different types of cells, we used the density gradient method to separate the different cell populations based on their density. Thus, we first obtained a mixture of lymphocytes and monocytes, which are special cells of the immune system. Then, we isolated the monocytes by magnetic separation. In this case, we use magnetic spheres that adhere specifically to the monocytes, which are then isolated thanks to a magnet.

We then proceeded with the construction of the skin substitute. For this, we had to recreate both the dermis and the epidermis. For the dermis, we made a fibrin gel, into which we incorporated the fibroblasts. Fibrin is a blood plasma protein and is involved in the formation of blood clots. In our study, we took blood plasma, incorporated the fibroblasts and mimicked the clotting process by adding calcium. We thus obtained a gel with embedded fibroblasts and clot-like characteristics. This became our dermal substitute.

Construction process of the skin equivalent.
Photograph: Communications Office.

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For the creation of the epidermis, we used fibrin gel as a support to seed keratinocytes in some cases, and keratinocytes and monocytes in others. These cultures were fed for 21 days since this is the time it takes for keratinocytes to grow and change their function, and thus form the epidermis.

The Potential of Monocytes in Skin Substitutes

After analyzing the skin substitutes, we found that the physical characteristics of the substitute were similar to those of human skin and the presence of keratinocytes and fibroblasts led the monocytes to become dendritic cells, i.e., cells of the immune system responsible for initiating the sensitization response. As expected, these were not found in cultures generated with keratinocytes alone. This result indicated that it is possible to use monocytes to obtain skin substitutes to study skin immunology.

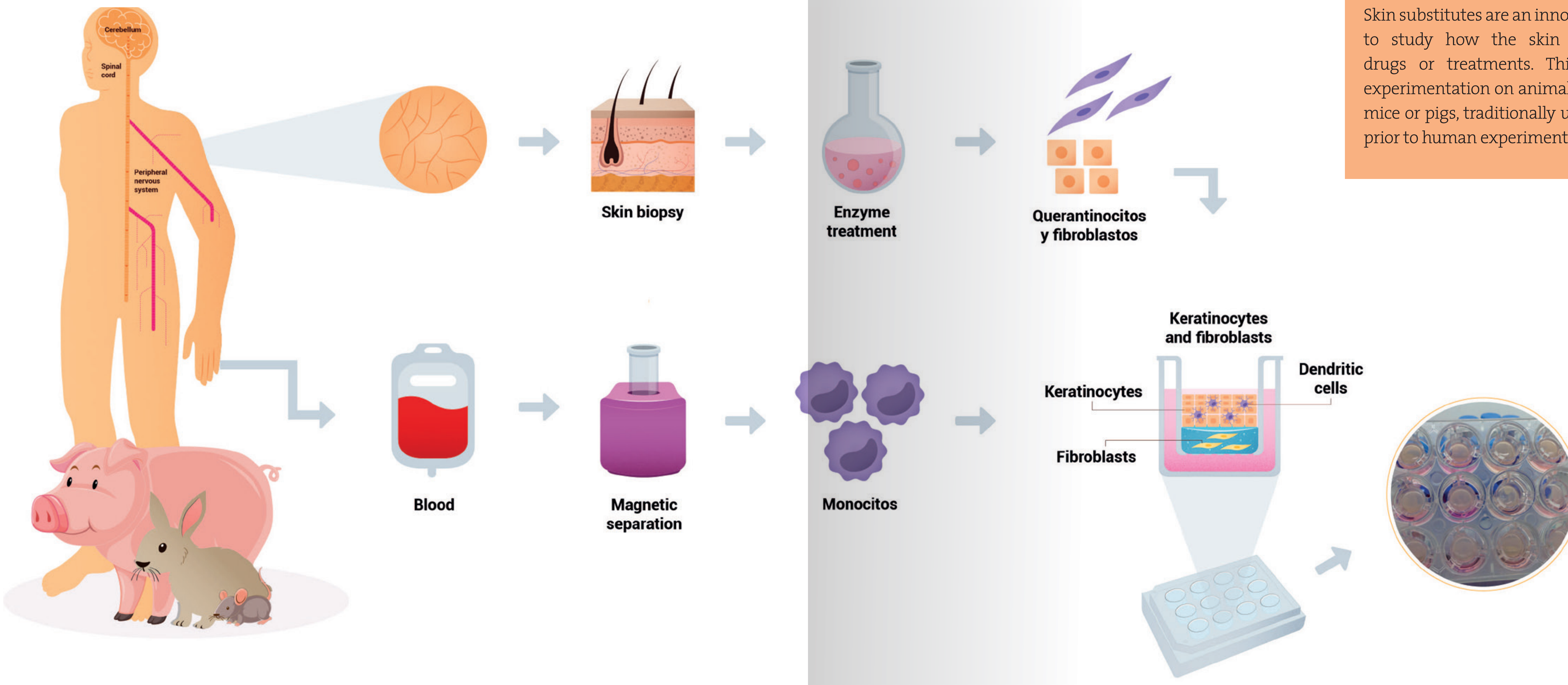
We then evaluated the ability of the skin substitutes to respond to sensitizing substances. For this, we applied formaldehyde and manganese chloride, two known sensitizers. At the same time, we applied saline as a control substance, which allowed us to check that the effects detected were not the result of other culture conditions.

When a sensitizer comes into contact with the skin, dendritic cells are activated and migrate to the lymph nodes. In doing this, they pass through the dermis. For this reason, we decided to analyze the location of these cells in our substitutes after exposure to the test substances. We observed that there was a migration of dendritic cells for the sensitizers. They exhibited a different location from that of the control.

The Promise of Skin Substitutes with Immune System Cells

The results of our study indicated that skin substitutes composed of keratinocytes and fibroblasts can promote the differentiation of monocytes in skin immune cells. This is probably because of molecules and factors released by keratinocytes and fibroblasts that "command" monocytes to become dendritic cells, most likely of different types. Additionally, these cells could have migrated through the fibrin gel in response to sensitization, which is close to what happens in human skin.

To our knowledge, this methodology has not been used before and represents a simpler method to obtain dendritic cells in substitutes or skin equivalents. Additionally, it highlights the possibility of obtaining different types of immune cells in a substitute and from a single precursor such as monocytes, which improves on current skin models.



Skin substitutes are an innovative alternative to study how the skin would react to drugs or treatments. This could replace experimentation on animals such as rabbits, mice or pigs, traditionally used in the stages prior to human experimentation.

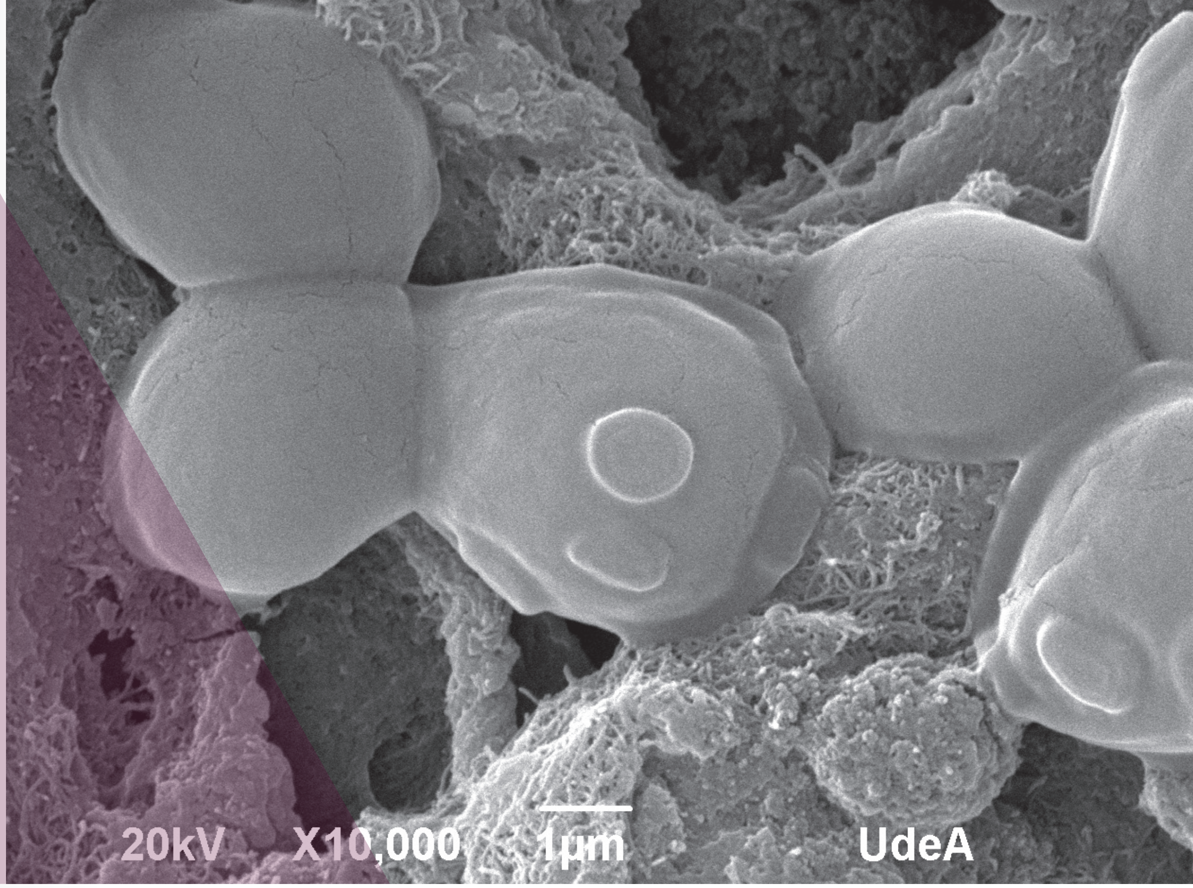
In addition to the immune system, skin substitutes have numerous applications in different fields. For example, apart from sensitizers, they can be used to evaluate irritating or corrosive substances, model diseases such as cancer or psoriasis (and thus develop possible treatments), study skin pigmentation by adding another type of cells called melanocytes and even as skin grafts for people who have suffered burns.

At present, commercially available human skin models have high costs and involve long import and legalization procedures to be used in Colombia. Besides, their transportation may result in a possible loss of product quality. Additionally, most are composed only of keratinocytes, which excludes their use in the field of immunology. Thus, with this project, we seek to obtain a local model that enables the development and commercialization of products in Colombia. X

Glossary

Dendritic cells: special type of immune cells that process antigens (molecules that induce an immune response) and present them to other cells of the immune system.
Enzyme: protein that accelerates the speed of chemical reactions in living beings.
Density gradient: fluid whose density changes and which is used in the separation of different types of cells by centrifugation.
Blood plasma: liquid portion of blood.
Lymph nodes: structures that filter substances and contain immune system cells.

Saccharomyces cerevisiae, from Wine to Electrochemical Sensors



Yeast cells arranged on oxidized multi-walled carbon nanotubes after modification of the printed carbon electrode. Photo: courtesy of the project.

Isabel Acevedo Restrepo
Chemist, PhD in pharmaceutical and food sciences.

Lucas Blandón Naranjo
Chemist, PhD in chemical sciences.

Members of the Electrochemistry line of the Interdisciplinary Group of Molecular Studies (GIEM), Faculty of Exact and Natural Sciences, Universidad de Antioquia.

The GIEM has designed an electrochemical sensor to measure the yeast that has given us wine, beer and bread. It has also helped us in the design of drugs and other applications. Although it may seem simple, the sensor is a technological development that opens doors for the study of the behavior of *Saccharomyces cerevisiae* using new methodologies.

In difficult days when a tiny creature, imperceptible to the naked eye, has sown terror and chaos throughout the planet and has forced us to rethink our daily lives, it is worth highlighting the work of those microorganisms that, on the contrary, have made our existence easier.

Within this microscopic universe, one yeast stands out: *Saccharomyces cerevisiae*. It could be considered the queen among the more than 1,500 species of yeast (of which approximately 80% have biotechnological applications) since it is known precisely for its wide use in different bioprocesses and its close relationship with humans. Since ancient times, we have been using this oval microorganism in the preparation of delicious elixirs such as wine and beer, exquisite foods such as bread, or in more complex and sophisticated applications such as the production of proteins and precursors for the manufacture of medicines.

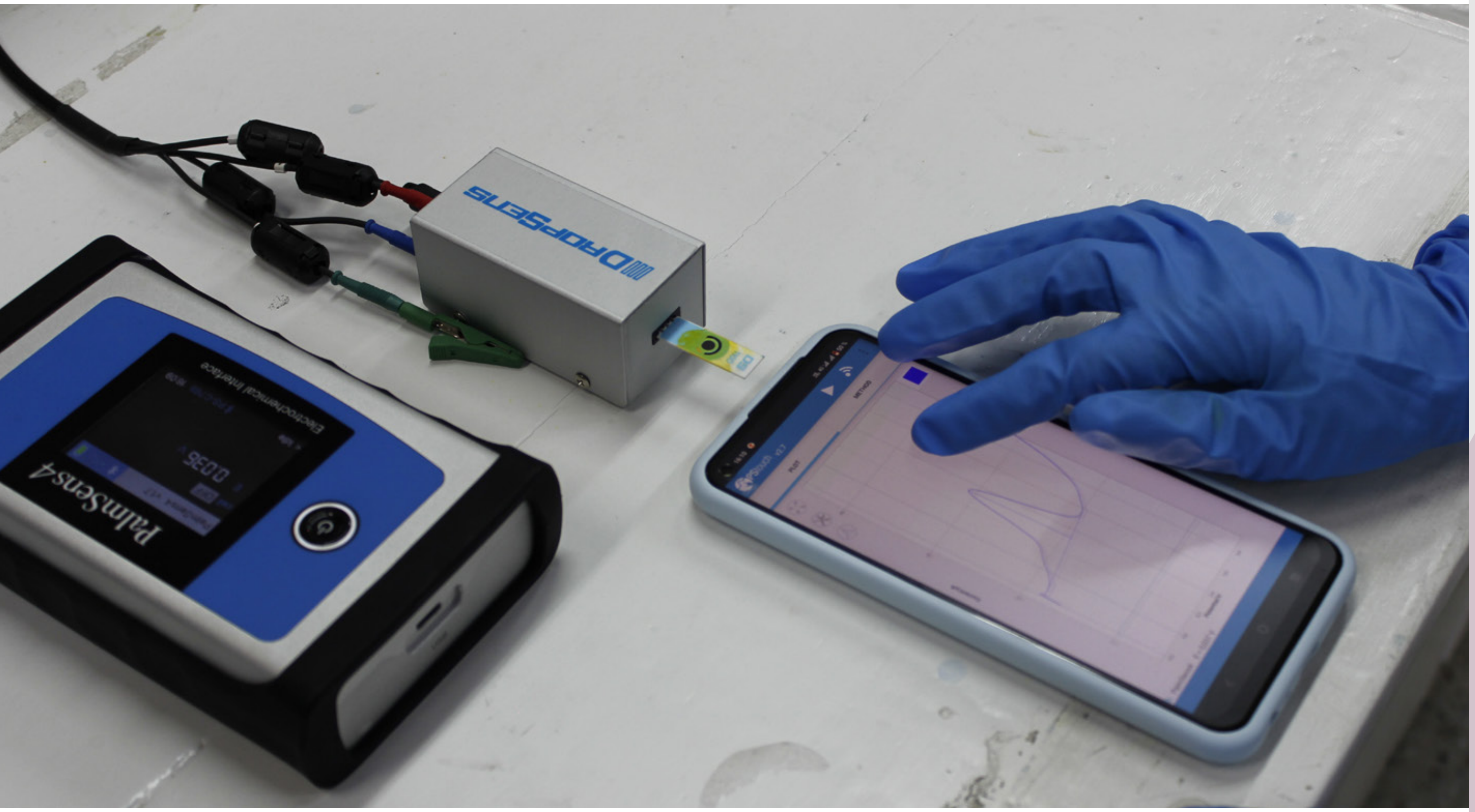


Photo: cortesía del proyecto.

Such has been the importance of *Saccharomyces cerevisiae* that even some anthropologists have come to consider the production of alcoholic beverages as the reason why primitive men decided to settle down and become farmers. They imagine that in about 6000 BC, curious gatherers tasted the contents of harvest containers where grapes fermented spontaneously. From then on, they preferred to consume the grapes transformed into that delicious elixir and enjoy its pleasant effects. Thus, fermentation prevailed and developed through different generations and cultures. During the period 4000-2000 BC, the Egyptians discovered how to make bread with yeast. Although at that time it was not clear how fermentations took place, it is now known that *Saccharomyces cerevisiae* is responsible for these biochemical processes.

This beneficial microorganism belongs to the kingdom *Fungi* and the family Ascomycota. It has an ellipsoid shape, and its size ranges between 5-10 millimeters. It is in constant interaction with us, and we can find it in different environments (in soil, trees, plants and fruits). It has also been, by far, the most studied eukaryote, so it has served to understand the biology of this type of cell.

Saccharomyces cerevisiae is of great biotechnological interest since it not only facilitates fermentation for food preservation but is also key to the development of different bioprocesses, which facilitates the production of high-value-added products such as proteins, amino acids, antibiotics, vaccines, cells, food additives, biopesticides and biofuels. *S. cerevisiae* is very useful for recognizing the mechanisms of certain diseases and for phytosanitary formulations, bioremediation and biocontrol. It is also used in the production of enzymes and different chemical substances. In addition, it has favored important advances in different fields such as cell biology, genetic engineering and biochemistry.

Given the number of applications that this yeast has, it is important to know aspects of its behavior during bioprocesses to guarantee their development and performance. This implies designing techniques that enable the control and monitoring of the yeast. Although there are many methodologies for these purposes, it is precisely at this point that *Saccharomyces cerevisiae* moves from barrels to electrochemical sensors.

The Challenge of Measuring a Tiny Living Thing

An electrochemical sensor is a device that allows the presence of a substance to be related to a variable, which can be an electrical current, voltage or conductivity. Electrochemical sensors are analytical tools that consist of a working electrode as an element of transduction and a chemical or biological recognition element. Transduction is the process of changing a response signal to a value that can be interpreted, for example, a digital thermometer that receives a thermal signal and transforms it into an electrical impulse that is "translated" into a number in degrees Celsius. The interaction between the recognition element and the analyte of interest results in a chemical change that is translated by the transduction element into an electrical signal, which can be monitored through different electroanalytical techniques. An example of this type of device is the blood glucose meter, which correlates the content of this molecule with the current generated by its reaction on the sensor. This current is translated into a numerical value that allows us to know whether glucose levels are adequate or whether we should take special measures to control them.

The versatility of electrochemical sensors makes them very advantageous since they offer many options: They enable decentralized, sensitive, selective, fast and low-cost measurements without the need for complicated sample pretreatment. Moreover, the sample size is small, even in the order of microliters (μL), which minimizes costs, as well as the use of reagents and contaminating materials.

Now, electrochemical sensors are usually designed to measure chemicals (glucose, oxygen, dopamine, etc.), but measuring microorganisms is another matter because they are living things! The challenge then is to imagine how a microorganism can cause a chemical reaction that results in an electrical response that can be measured and correlated with the presence of the microorganism. This seems complicated, but if we consider that all living things are reactors that produce billions of chemical reactions per minute, it makes sense that some of these chemicals can be measured by the sensor and, in turn, correlated with the number of microorganisms present in a medium.

A Sensor Tailored to a Yeast

The idea of studying a microorganism from the electrochemical point of view arose thanks to the work carried out in the Electrochemistry line of the Interdisciplinary Group of Molecular Studies (GIEM). This experience made it possible to determine, through the design of electrochemical sensors, biological molecules such as NADH, a biomolecule present in about 300 metabolic processes including ethanolic fermentations and respiration. Mangiferin was another molecule discovered. It is one of the main metabolites found in mango and has antioxidant properties.

The working electrode can be of different materials, configurations and sizes. Currently, printed electrodes (SPE) stand out. They are developed by printing different materials, such as graphite, on a substrate that is generally plastic or ceramic. The resulting electrode integrates the necessary elements to carry out the electrochemical determinations on the same platform. SPEs have become a striking electrochemical alternative because of their versatility and multiple advantages: They are easy to set up, simple, portable, highly sensitive and disposable. Moreover, their response is fast, and they operate at room temperature with no pre-polishing required. These are just a few advantages. In general, the success of SPEs lies in the possibility of combining their ease of modification, operation and portability with simple and inexpensive electrochemical methodologies.

To modify the electrodes, there are different recognition elements, among which oxidized multi-walled carbon nanotubes stand out. Their physicochemical characteristics improve the immobilization and chemical reactivity of biomolecules, apart from increasing sensitivity and promoting electron transfer. The modification of the working electrodes is carried out to confer selectivity and sensitivity, improve detection and quantification limits, and even obtain signals from species that are not electroactive in principle.

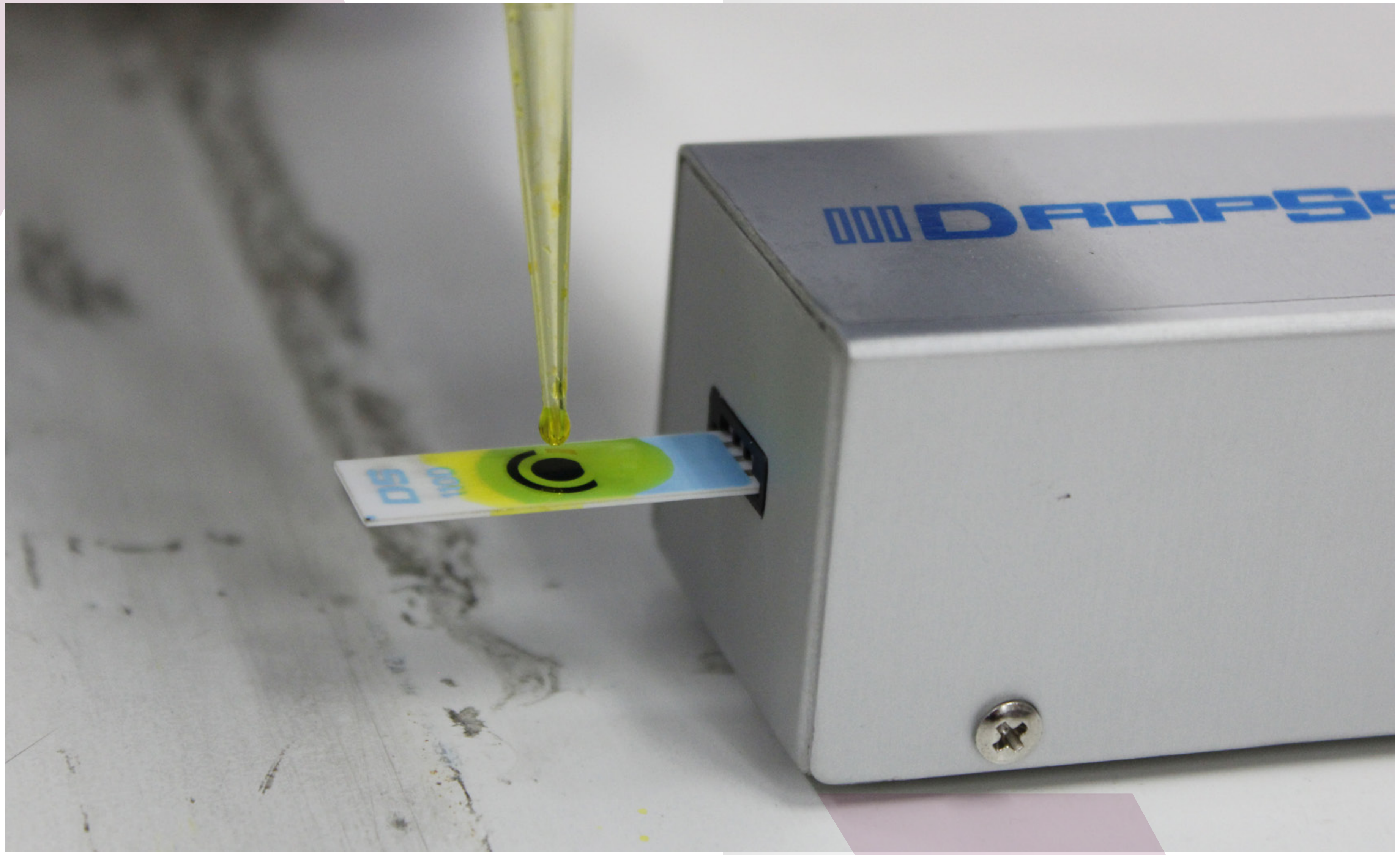


Photo: cortesía del proyecto.

Within the project Electrochemical Study of Fermentations of Industrial Interest, carried out in the GIEM group, a printed carbon electrode was used. Its surface was modified by the addition of carbonaceous nanomaterials and Nafion® (a synthetic polymer with ionic properties). This allowed them to obtain an electrical signal that indicated the presence of yeast, which proved that the electrochemical method can be a valid alternative, especially considering the simplicity and speed of the method compared to other methodologies commonly used to quantify yeast.

These results, besides being useful from the point of view of control and monitoring of bioprocesses, are very interesting if we consider that electrochemical determinations are usually performed on organic or inorganic molecules. However, this result shows that it is also possible to study the behavior of living entities such as *S. cerevisiae*. This is very promising for a large number of processes in which the work with microorganisms is of interest, specifically with *S. cerevisiae*.

In the meantime, while we are studying the behavior of *Saccharomyces cerevisiae* in isolation and during bioprocesses, such as ethanolic fermentations, using the designed electrochemical sensor and electroanalytical techniques, let's enjoy a good book and a delicious glass of wine. **X**

Glossary:

Bioprocess: process in which living microorganisms are involved to bring about physical or chemical changes.

Analyte: chemical substance that can be detected, identified or quantified when the chemical analysis of a specific sample is performed.

Working electrode: place where an electron transfer occurs in an electrochemical reaction.

Sensitive Plant and Horsetail:

Roadside Plants with Antibacterial Potential

Mauricio Esteban Sánchez
Chemical engineer, MSc in engineering, mauricio.sanchez@udea.edu.co

Diana María Trujillo
Chemical engineer, dmaria.trujillo@udea.edu.co

Process Simulation, Design, Control and Optimization Research Group (SIDCOP) of the Chemical Engineering Department of Universidad de Antioquia.

The frequent appearance of strains of microorganisms with antibiotic resistance generates an increase in bacterial infections and diseases that are difficult to treat. This adds to the need to develop alternatives based on plant extracts to prevent and combat them.

Closed sensitive plant.
Photo: courtesy of the project.

Microorganisms are the most primitive and abundant beings that exist on Earth. They are found in air, water and soil; they actively participate in the functioning of biological systems and are in permanent interaction with humans, animals, plants and the environment they inhabit.

Germs are those microbes that harm human health: viruses, fungi, protozoa and bacteria, among which are *Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus).* The former is found in the intestines of humans and animals, the environment and sometimes in untreated food and water. However, there is a variety that can cause severe gastrointestinal conditions, urinary tract infections, respiratory diseases and bloodstream infections in humans. S. aureus causes infections by penetration of the bacterium from the skin into deep tissues when skin, traumatic or surgical injuries occur. Such infections are usually suppurative and tend to produce abscesses. Due to its wide versatility, it can cause a broad spectrum of diseases, from minor skin infections to serious invasive infections such as osteomyelitis and bacteremia. It can also cause infections of the central nervous system, respiratory tract and urinary tract, not to mention toxic shock syndrome and gastrointestinal infections. Thus, it is evident that we are exposed to these microorganisms and their risks daily.

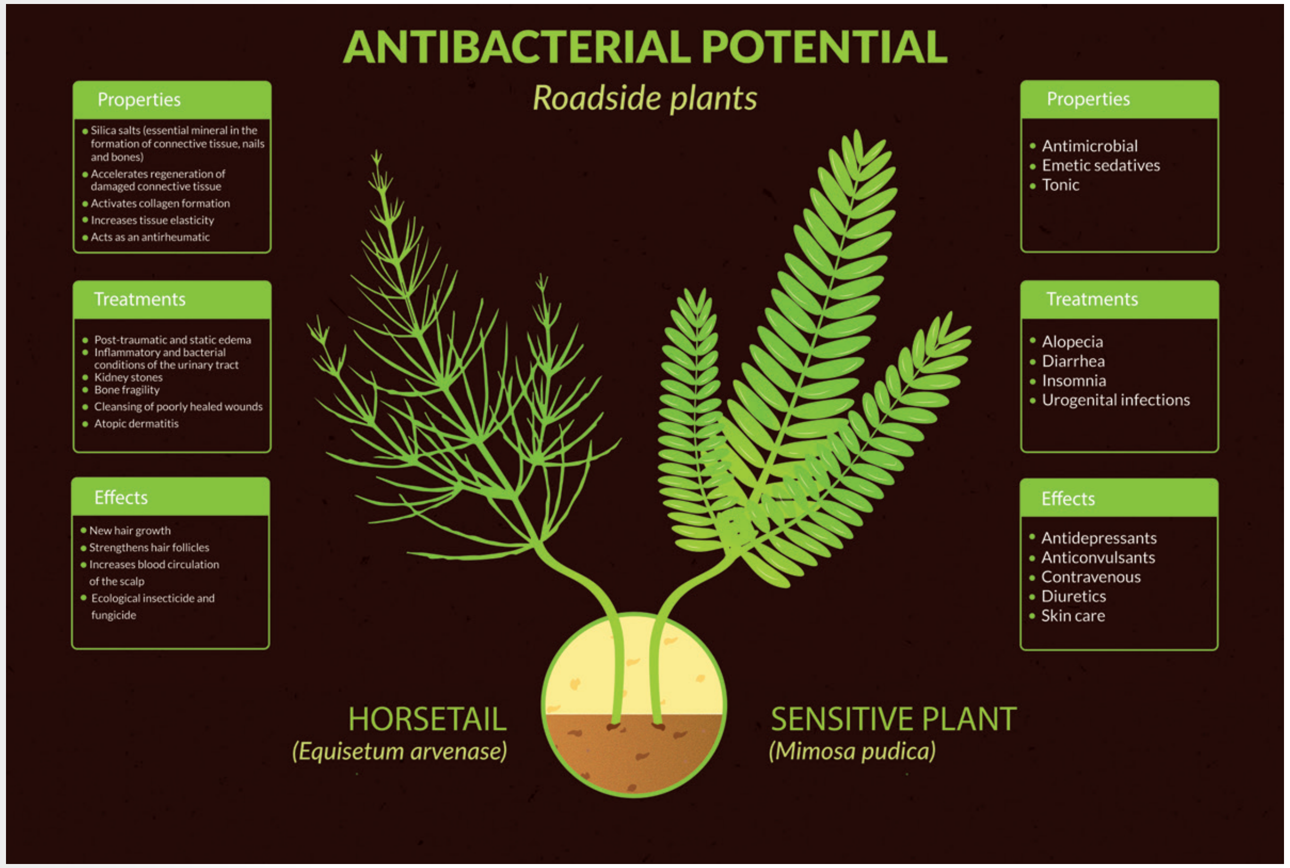
The antimicrobial property of substances extracted from some plants is of great importance in medical, cosmetic, food and pest control applications, among others. This property is attributed to compounds such as flavonoids, tannins, phytoalexins, pyrethrins, musanolones, terpenes and essential oils. These are known as secondary metabolites. Plants produce them through sequences of chemical reactions that occur in a specific order called secondary metabolism. Although these metabolites do not have vital functions, they serve as protection from the sun's ultraviolet rays and as a defense against predators and pathogens. They also attract pollinating insects and vary with the plant's interactions with the environment.

Historically, natural products have played an important role in improving human health and people's quality of life. Although the popularity of herbal medicine registered a marked decline after the introduction of chemical drugs used in conventional medicine, herbal medicines are once again gaining interest due to their natural, environmentally friendly attributes and the possibility of achieving true relief of disease without resorting to synthetic substances. Harmful side effects, the high cost of other forms of treatment and their limited availability for poor populations in remote areas are also reasons for the increasing use of herbal medicine, which has more than 3,000 years of tradition in countries such as India and China.

Colombia is a country with a great diversity of plant species, many of them considered weeds. However, their secondary metabolites have very beneficial properties for human beings, which generates an opportunity to generate high environmental, social and economic growth impacts.

Based on these opportunities, Universidad de Antioquia's SIDCOP group conducted a study with plants commonly known as horsetail (*Equisetum arvense*) and sensitive plant (*Mimosa pudica*) to determine whether ethanolic extracts and mixtures have antibacterial activity against strains of *E. coli* and S. aureus. Thus, we explored their potential to develop new high-value products with eventual applications in the agricultural, cosmetic, food and medical fields. This project focused on obtaining alcoholic extracts under specific conditions of temperature, alcohol/plant material ratio, contact time, plant origin and mixtures of different extracts at various compositions to determine their possible effect on inhibiting the growth of *E. coli* and S. aureus bacteria. We do not know of other studies that have reported the test conditions of this work.

For this study, the plant material was collected in the municipality of Gigante, Huila. It was dried, cleaned and ground. Then, the alcoholic extraction of the dried material was performed by putting the dried leaves of each species in 96% ethyl alcohol for 72 hours at room temperature. The extracts were purified by heating to evaporate the solvent at atmospheric pressure (simple distillation) and in the presence of a vacuum (vacuum distillation). The extracts and their mixtures were subjected to qualitative phytochemical tests to identify secondary metabolites. Disc-plate antibiogram tests were performed to determine their potential to inhibit bacterial growth. Microdilution tests in broth were also carried out to find the minimum inhibitory concentration against *E. coli* and *S. aureus* strains. The antibiotics gentamicin and clindamycin were used as positive controls to compare their effectiveness.



The phytochemical screening of the ethanolic extracts of horsetail and sensitive plant verified the presence of secondary metabolites such as flavonoids, saponins, tannins and triterpenes.

The results of this study determined the antibacterial potential of different extracts of *Mimosa pudica*, *Equisetum arvense* and their mixtures against *E. coli* and *S. aureus* for possible uses in the development of new products of high economic value in the pharmaceutical, food, nutraceutical, cosmetic and cosmeceutical industries. The tests showed that the mixtures of the extracts have an effect that enhances antibacterial activity since they showed the best results in growth inhibition for the two bacterial strains.

Based on the results, a very interesting exploratory window opens up for multiple applications of mixtures of sensitive plant

Cola de caballo.
Foto: cortesía del grupo

and horsetail extracts, which could contribute to the care of people and pest control in organic agriculture. Among the possible applications, we can mention:

• Preservatives for perishable foods, such as meats, processed cheeses and canned products.
• Easily digestible antibiotics to treat urinary, respiratory, gastrointestinal and skin infections; antibiotics unlikely to generate resistance.
• Preservatives for cosmetic products.
• Active compounds in cosmeceutical and nutraceutical products. X

Glossary

Simple distillation: thermal technique to separate mixture components.
Vacuum distillation: distillation process at reduced pressure that seeks to separate mixtures at low temperatures.
Disc-plate antibiogram: method used to determine bacterial sensitivity to antimicrobial agents.
Cosmeceuticals: industry that produces cosmetics with much more concentrated active ingredients than classic cosmetics, so they have a deeper penetration and require prescription and follow-up by a dermatologist or skin care specialist.
Nutraceutical: industry that produces food of animal or vegetable origin that benefits health and nourishes the body.
Strains: group of microorganisms of the same species from a specific sample or cell.

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