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Production of a microemulsion of American genipa l. (rubiacea) bark extract and the antioxidant and antiparasitic activities

Produção de uma microemulsão de genipa americana l. (rubiácea) extrato da casca e as atividades antioxidante e antiparasitária

Producción de una microemulsión de genipa americana l. (rubiacea) extracto de corteza y las actividades antioxidantes y antiparasitárias

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Pietra Alexia Lima dos Santos
ORCID: https://orcid.org/0000-0002-7126-2265
Federal University of Sergipe, Brazil
E-mail: pietra-alexia@hotmail.com
Mário Rodrigues Pereira da Silva
ORCID: https://orcid.org/0000-0002-2444-5798
Federal University of Sergipe, Brazil
E-mail: Mariosilvarp29@gmail.com
Ludmila Cruz dos Santos
ORCID: https://orcid.org/0000-0001-6503-9908
Federal University of Sergipe, Brazil
E-mail: ludmilahcruz@gmail.com
Igor Adriano de Oliveira Reis
ORCID: https://orcid.org/0000-0002-4941-9932
Federal Institute of Sergipe, Brazil
E-mail: reisigoradriano@gmail.com
Clêrton Magno Rocha Santana Pereira
ORCID https://orcid.org/0000-0002-9191-2448
Federal University of Sergipe, Brazil
E-mail: clerton.rocha@hotmail.com
Jeison Saturnino de Oliveira
ORCID: https://orcid.org/0000-0003-3035-2560
Federal University of Sergipe, Brazil
E-mail: jeison_fisioterapia@yahoo.com.br
Waldecy de Lucca Junior
ORCID: https://orcid.org/0000-0003-2916-9101
Federal University of Sergipe, Brazil
E-mail: wluccajr@academico.ufs.br
Américo Azevedo de Souza
ORCID: https://orcid.org/0000-0002-2006-9107
Federal University of Sergipe, Brazil
E-mail: americosouza_bio@yahoo.com
Samuel Bruno dos Santos
ORCID: https://orcid.org/0000-0001-6420-406X
Federal University of Sergipe, Brazil
E-mail: samuelbruno@gmail.com
Charles dos Santos Estevam
ORCID: https://orcid.org/0000-0001-6940-6891
Federal University of Sergipe, Brazil
E-mail: cse.ufs@gmail.com

Abstract

Genipa americana L. is a plant native to the Americas, popularly known in Brazil as Jenipapeiro, and is chemically characterized by the presence of genipin, which has pharmacological actions such as anti-inflammatory and antioxidant. The bark is used for its antiulcerogenic and antidiarrheal activities. Research developed for chemical knowledge have shown the plant is of interest for the production of efficient drugs such as antioxidants and antimicrobials. Free-living parasites, including those of the genus *Acanthamoeba*, have aroused interest and the study of plants used in folk medicine is a strategy for the discovery of conventional treatments to the infections caused by this parasite to be used pure or in preparations such as microemulsions (ME), which offer advantages related to the

release of drugs and the absorption of the active ingredient. The aim of the study was to evaluate antiparasitic and antioxidant activities as well as to obtain a ME from the hydroethanolic extract (HEE) of *G. americana*. The DPPH free radical reduction method was used to evaluate the antioxidant activity of HEE, which showed an inhibition percentage (PI) of 47.82%. The antiparasitic activity of the HEE using *A. castellanii* trophozoites showed a PI of 80% at the highest concentration tested (9 mg mL^{-1}). The ME containing 10% HEE was characterized for its droplet size (18.33 µm), pH (5.68) and polydispersity index (0.19). Therefore, the HEE presented antioxidant and antiparasitic effects, while its microemulsion showed potential to disperse particles.
Keywords: *Genipa americana L.*; Anti-parasitic; Antioxidant activity.

Resumo
A Genipa americana L. é uma planta nativa das Américas, conhecida popularmente no Brasil como Jenipapeiro, e é caracterizada quimicamente pela presença da genipina, que possui ações farmacológicas como anti-inflamatória e antioxidante. A casca é usada por suas atividades antiulcerogênicas e antidiarréicas. Pesquisas desenvolvidas para conhecimento químico têm mostrado que a planta é de interesse para a produção de fármacos eficientes como antioxidantes e antimicrobianos. Parasitos de vida livre, incluindo os do gênero Acanthamoeba, têm despertado interesse e o estudo de plantas utilizadas na medicina popular é uma estratégia para a descoberta de tratamentos convencionais para as infecções causadas por este parasita a serem utilizados puros ou em preparações como microemulsões (ME), que oferecem vantagens relacionadas à liberação de fármacos e à absorção do princípio ativo. O objetivo do estudo foi avaliar as atividades antiparasitária e antioxidante, bem como obter uma EM do extrato hidroetanólico (HEE) de G. americana. O método de redução de radicais livres DPPH foi utilizado para avaliar a atividade antioxidante do HEE, que apresentou um percentual de inibição (PI) de 47,82%. A atividade antiparasitária do HEE utilizando trofozoítos de A. castellanii apresentou IP de 80% na maior concentração testada (9 mg mL-1). O ME contendo 10% de HEE foi caracterizado quanto ao tamanho de gota (18,33 µm), pH (5,68) e índice de polidispersão (0,19). Portanto, o HEE apresentou efeitos antioxidantes e antiparasitários, enquanto sua microemulsão mostrou potencial para dispersar partículas.
Palavras-chave: *Genipa americana L.*; Antiparasitária; Atividade antioxidante.

Resumen
Genipa americana L. es una planta originaria de las Américas, conocida popularmente en Brasil como Jenipapeiro, y se caracteriza químicamente por la presencia de genipina, que tiene acciones farmacológicas como antiinflamatoria y antioxidante. La corteza se utiliza por sus actividades antiulcerogénicas y antidiarreicas. Las investigaciones desarrolladas para el conocimiento químico han demostrado que la planta es de interés para la producción de medicamentos eficientes como antioxidantes y antimicrobianos. Los parásitos de vida libre, incluidos los del género Acanthamoeba, han despertado interés y el estudio de plantas utilizadas en la medicina popular es una estrategia para el descubrimiento de tratamientos convencionales a las infecciones causadas por este parásito para ser utilizados puros o en preparados como microemulsiones (ME), que ofrecen ventajas relacionadas con la liberación de fármacos y la absorción del principio activo. El objetivo del estudio fue evaluar las actividades antiparasitaria y antioxidante, así como obtener una EM a partir del extracto hidroetanólico (HEE) de G. americana. Se utilizó el método de reducción de radicales libres DPPH para evaluar la actividad antioxidante del HEE, el cual mostró un porcentaje de inhibición (PI) de 47,82%. La actividad antiparasitaria del HEE utilizando trofozoítos de A. castellanii mostró una IP del 80% a la concentración más alta probada (9 mg mL-1). La ME que contenía 10 % de HEE se caracterizó por su tamaño de gota (18,33 µm), pH (5,68) e índice de polidispersidad (0,19). Por lo tanto, el HEE presentó efectos antioxidantes y antiparasitarios, mientras que su microemulsión mostró potencial para dispersar partículas.
Palabras clave: *Genipa americana* L.; Antiparasitario; Actividad antioxidante.

1. Introduction

Sometimes, communities and ethnic groups only have the knowledge about medicinal plants as a therapeutic resource and their use is ancient for the treatment and cure of diseases. In Brazil, it is possible to observe the gradual and significant growth in the use of plants as a medicinal alternative (Santi et al., 2014), with the demand for bioactive substances as a highlight. Today, it is common to find medicinal plants in popular markets and open markets in various regions of the country (Maciel, Pinto & Veiga jr., 2002). The use of these natural resources has been recommended, since public health has been facing obstacles regarding the increased number of resistant microorganisms. For the most part, medicinal plants have considerable biological effects, which can be used as guides for the isolation and production of phytotherapics and phytopharmaceuticals (Barreiro; Fraga & Araújo Júnior, 2007), besides economic advantages that allow for them to be seen as promising alternative treatments of infections.

Free-living parasites are of great interest because some diseases are difficult to treat with the substances available on the market. Free-living amoebas are protozoa widely distributed in nature, with the genus *Acanthamoeba* as one of the most studied because they are responsible for several pathologies in humans and animals, such as granulomatous amoebic encephalitis and amoebic keratitis (Marciano-Cabral & Cabral, 2003; Schuster & Vivesvara, 2004). The treatment of granulomatous amoebic encephalitis is comprised by a combination of pharmacological substances, such as isothionate, sulfadiazine, flucytosine, fluconazole and itraconazole (Santos et al., 2016), while amoebic keratitis protozoa is resistant to the drugs currently used, leading to recurrences of the disease and highlighting the need for new efficient drugs for these situations (Dart et al., 2009). Therefore, the analysis of plants used in folk medicine is a strategy for the discovery of possible conventional treatments.

Genipa americana L. belongs to the Rubiaceae family and is popularly known as Jenipapeiro, being distributed along the Brazilian coast and Atlantic Forest. It can also be found in South and Central America, Africa and Asia (Ueda et al., 1991; Kumar et al., 2016). The evaluation of the chemotaxonomic profile of the plant family showed the presence of glycosylated iridoids in several species, leading to the conclusion that these metabolites are probably present in most subfamilies of Rubiaceae (Hiroyuki et al., 1988). *G. americana* is chemically characterized by the presence of genipin, an iridoid isolated from fruits of the species in Brazil (Djerassi, 1960), which has anti-inflammatory, antioxidant and antiangiogenic activities as pharmacological properties (Almog et al., 2004; Byung-Chul et al., 2005; Koo et al., 2004). Its popular use is marked by several purposes (Barbosa, 2008), among them antiulcerogenic, antidiarrheal and cathartic activities, mainly related to its bark.

The decocted leaves of *G. americana* have antidiarrheal and antisyphilitic activities, and when macerated they treat fever. Its roots have purgative and antigonorrheic powers, while its fruit acts as an antiasthmatic, antianemic and diuretic tonic. Alves et al. (2008) reported the use of the plant leaves and bark by the population as blood purifier. These biological activities are mainly explained by the presence of iridoids and other secondary metabolites such as coumarins, flavonoids, alkaloids and terpenoids; which are well-known for several biological activities, such as antitumor, anti-inflammatory, antibacterial, antiparasitic, among others (Coutinho et al., 2009; Middleton et al., 2000; Vunda et al., 2012).

Microemulsion (ME) systems offer advantages related to the release of drugs, favoring the absorption of the active ingredient, increasing the therapeutic efficacy, and consequently reducing the administered dose and minimizing the potential side effects of the drugs (Dantas et al., 2010; Formariz et al., 2005). ME is a thermodynamically stable, isotropic and transparent system composed of two immiscible liquids (water and oil), which are stabilized by the addition of surfactants these are located at the oil/water interface (Formariz et al., 2005). According to Damasceno et al. (2011), the formation of ME differs from emulsions because in addition to the system being thermodynamically stable and transparent it has a nanometric droplet size. ME orientation for Oil/Water (O/W) or Water/Oil (W/O) systems is dependent on the physicochemical properties of the surfactant and oil and between the water/oil proportions (Constantinides et al., 1994; Oliveira et al. al., 1997).

The amoebicidal activity of the hydroethanolic extract (HEE) of the inner bark of *G. americana* L. has not been reported in the literature as well as the use of microemulsion as a product. Thus, the present study aimed to evaluate the antiparasitic and antioxidant activity of the HEE, which was incorporated in a microemulsion system, against the trophozoites of *Acanthamoeba castellanii*.

The present study had the technological objective of obtaining a microemulsion based on the hydroethanolic extract of *G. americana* with possible antiparasitic activity, initially evaluating the antioxidant potential of the crude sample of the vegetable bark. The general objective of the present study was to evaluate the biological potentials of the hydroethanolic extract of *G. americana* L. bark, represented in this study by the antioxidant and antiparasitic activities. In addition, this study also aimed to obtain and characterize a microemulsion system based on the hydroethanolic extract of *G. americana* L.

2. Methodology

Harvest and identification of the plant material

The bark of the plant was collected in the municipality of São Cristóvão, 25 km from the capital Aracaju of the Sergipe state. Brazil. The coordinates for the harvesting were latitude 10º58'37.5"S and longitude 37°13'05.8"W, predominantly in the Atlantic Forest biome. The plant exsiccate was deposited in the Herbarium of the Biology Department of the Federal University of Sergipe under voucher number ASE 37.908. Its botanical identification was carried out by taxonomist Dr. Ana Paula Nascimento Prata.

Preparation of the hydroalcoholic extract

The bark of *G. americana* (1.2 kg) was initially subjected to drying in an oven at 45°C, then ground in a knife mill to a powder. The material obtained was placed in a glass flask with the extracting solvent (ethanol 90% v/v in water) at 25°C. Three successive extractions were carried out at 10 day intervals (extraction method modified from Cechinel Filho & Yunes, 1998). The extract was filtered and concentrated in a rotary evaporator under reduced pressure at 60°C to give 66.5 g of the hydroethanolic extract (HEE) with a yield of 5.54%.

Phytochemical Prospecting

For the determination of the secondary metabolite groups, classical methods and chemical reactions were applied to the HEE (5 mL, 1 mg mL^{-1}), including pH-related color variation by adding sodium hydroxide (3 mol L^{-1}) and/or hydrochloric acid (1 mol L^{-1}), acid-base reactions followed by heating, ferric chloride (1 mol L^{-1}) precipitation, magnesium in concentrated hydrochloric acid, and reactions such as Lieberman-Buchard (with and without foam formation) and Dragendorff. The organic extract was submitted to the phytochemical screening following the procedures described in the literature by Wagner & Bladt (1995) and Matos (1997).

Antioxidant activity of the HEE using the DPPH free radical method

The antioxidant effect of the HEE from *G. americana* L. bark against the DPPH radical was analyzed by spectrophotometry at 515 nm (SOLER-RIVAS et al., 2000). The consumption of the radical was analyzed for 60 minutes using a spectrophotometer. The reduction of DPPH (%) was calculated by the formula [(ADPPH)-(ASample)/ADPPH] x 100%, where ADPPH = absorbance of the control DPPH and ASample = absorbance of the DPPH in the presence of the sample. The CE_{50} was calculated from the graph created using the HEE concentration (5 a 25 µg mL^{-1}) x remaining DPPH (%). The antioxidant activity index (AAI) was calculated by dividing the value of the final concentration of the sample by the EC_{50} at 60 min and classified as weak when AAI < 0.5, moderate when 1 > AAI > 0.5, strong when 2 > IAA > 1 and very strong when IAA > 2 (Scherer & Godoy, 2009). Gallic acid **(**100 µg mL^{-1}) was used as a positive control. The reaction was initiated by adding an aliquot of the extract in spectrophotometric cuvettes containing a methanol solution of DPPH (3 mL; 40 µmol L^{-1}) to obtain the final concentrations for use in the standard curve of DPPH.

Antiparasitic activity

For the antiparasitic analysis of the *G. americana* L. bark HEE, trophozoites of *Acanthamoeba castellanii* were used. They were maintained in the Laboratory of Entomology and Tropical Parasitology of the Federal University of Sergipe in PYG medium at room temperature. For the assay, 8 x 10^4 trophozoites in logarithmic phase of growth were used, which were distributed in 24 wells cell culture plates containing 2 mL of PYG culture medium per well plus the HEE in different concentrations.

From a HEE solution (20 mg mL^{-1}), serial dilutions were made directly in the wells of the plates containing the culture medium and the trophozoites to give the final concentrations of 1, 3, 4, 5, 7, and 9 mg mL^{-1}. Each concentration was performed in triplicate and the assay was done three times. The positive control was made with ameba plus PYG culture medium without the HEE also in triplicate and in three replications. The amoebas were exposed to the HEE for 24 h, after which the plates were placed on ice for 20 min so that the parasites could detach from the plate materials. After homogenization, an aliquot of 12 μL of the well content was deposited in a Neubauer chamber for counting.

To calculate the concentration of HEE to give a 50% cell growth inhibition (CI_{50}), nonlinear regression was done using the percentages of cell viability and growth inhibition obtained from the Neubauer chamber counts. A R^2 = 0.93 was obtained.

Characterization of the microemulsion

Microemulsion (ME) preparation

From the Pseudoternary Phase Diagram (PTPD), it was possible to obtain points for the microemulsion formulation leading to transparency, homogeneity and optical isotropy. The choice of points had as first phase a buffer phosphate solution of pH 7.4 (68.0%), and as second phase Tween 80 (12.3%) and oleic acid (19.7%). The first phase was shaken for 20 min, while the second was stirred for 3 min, and both phases were magnetically agitated. Subsequently, the two phases were joined followed by sonication and ultrasound bath processing to finish the production of the ME (Silveira, 2009). A 20 mL of the vehicle formulation was obtained.

The dry HEE was incorporated directly into the microemulsions at 5 and 10 mg mL^{-1} at a controlled temperature of 25ºC under magnetic agitation. After homogenization, the microemulsified extract was centrifuged and 100 μL of the supernatant was removed and solubilized in ethanol for spectrophotometric readings at 279 nm. From this, the concentrations that were actually incorporated into the ME (CERA, 2001) were determined, which gave a 20 mL of the microemulsion containing the Hee at 10%.

pH determination

The pH of the microemulsions was determined using a digital potentiometer Phtek PH 3B with glass electrode and temperature sensor, which were previously calibrated. The electrode was placed directly in the formulation of the microemulsified system.

Polydispersity Index

This parameter was analyzed by dynamic light scattering (DLS) using a Zetasizer Nano System ZS (Malvern-UK) equipment located in the Colloidal Systems Core laboratory of the Tiradentes University. The vehicle microemulsion formulation was analyzed by putting a sample in a glass cell with 1 cm optical path. The measurements were performed at 25ºC.

Polarized Light Microscopy

To identify the formulation isotropy, polarized light microscopy (PLM) was performed, where a drop of the microemulsions was transferred to a glass slide, after the minimum time for the balance of the formulation had elapsed. The slide was then covered by a thinner glass cover and analyzed over polarized light. For this purpose, an Olympus BX-51 microscope equipped with a LC Color Evolution digital camera (PL-A662) and PixeLINK image analyzer software was used. It was located in the Multiuser Laboratory of the Physics Department of the Federal University of Sergipe.

3. Results and Discussion

Phytochemical Prospection

From the classical reactions for the phytochemical screening of the bark HEE from *G. americana*, it was possible to identify the presence of free steroids, flobabenic tannins, flavones, flavonols and saponins (Table 1). These metabolites are considered of great importance for the biological activities of the plants, especially antimicrobial and anti-inflammatory activities.

Table 1 - Phytochemical screening of the hydroethanolic extract from the bark of *G. americana* L.

SECONDARY METABOLITE CLASSEES	HYDROETHANOLIC BARK EXTRACT
Alkaloids	-
Anthocyanidins	-
Anthocyanins	-
Catechins	-
Chalcones and Aurones	-
Steroids	+
Phenols	-
Flavanones	-
Flavanonols	-
Flavones	+
Flavonoids	+
Flavonols	+
Leucoanthocyanidins	-
Quinones	-
Saponins	+
Flobabenic tannins	+
Triterpenoids	-
Xanthones	-

Present (+); Absent (-). Source: Santos (2022).

It was noted the presence of phenolic compounds, including tannins and flavonoids, which are reported in the literature as having antioxidant, antibacterial, antifungal and antiprotozoal activities, as well as antiproliferative, antiulcerogenic and anticarcinogenic actions (Muschietti & Martino, 2009). In addition, tannins act on tissue healing (Mello & Santos, 2001) and steroids participate in the development and control of the human reproductive system, being precursors of vitamin D and anti-inflammatory agents (Robbers et al., 1997).

Antioxidant activity – DPPH

The quantitative evaluation of the HEE antioxidant activity was performed according to the methodology described by Soler-Rivas et al. (2000). This methodology led to the determination of parameters related to the antioxidant activity of the extract against the stable radical 2,2-diphenyl-1-picrylhydrazil (DPPH) in a methanolic solution. The radical consumption is monitored by the decrease in the absorbance of the DPPH media in a UV-VIS spectrophotometer for 60 min, at times of 1, 5, 10, 20, 30, 40, 50 and 60 min. The CE_{50} is the concentration of extract to reduce a free radical by 50%, while AAI (antioxidant activity index) will show how strong the antioxidant is (Scherer & Godoy, 2009; Sousa et al., 2007).

Results for the quantitative evaluation of the HEE and gallic acid antioxidant activities are presented in Table 2. It can be seen the HEE presented an IAA > 2, which differs from the positive control AG, but both results are in the range of values

considered very strong, and consequently lead to a very evident antioxidant activity. There is a significant ($p<0.05$) difference between the percentage of inhibition for the HEE (47.82%) compared to the positive control gallic acid (97%). In the work by PORTO et al., 2014, a reduction of up to 58% of the DPPH radical was observed when the extract of the jenipapo fruit was analyzed. Studies in the literature correlate the content of phenolic compounds in an extract to its antioxidant power, as well as the chemical variability among the substances that can act synergistically in the sample, causing the antioxidant effect.

Table 2 - Antioxidant activity of the hydroethanolic extract (HEE) from *G. americana* bark and positive control gallic acid (GA) in the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay.

SAMPLE	ANTIOXIDANT PARAMETERS		
	AAI	**CE_{50*}** (μg mL^{-1})	**IP** (%)
HEE	3.13 ± 0.03a	12.78c	47.82a
GA	2.90 ± 0.05b	13.81c	7.00b

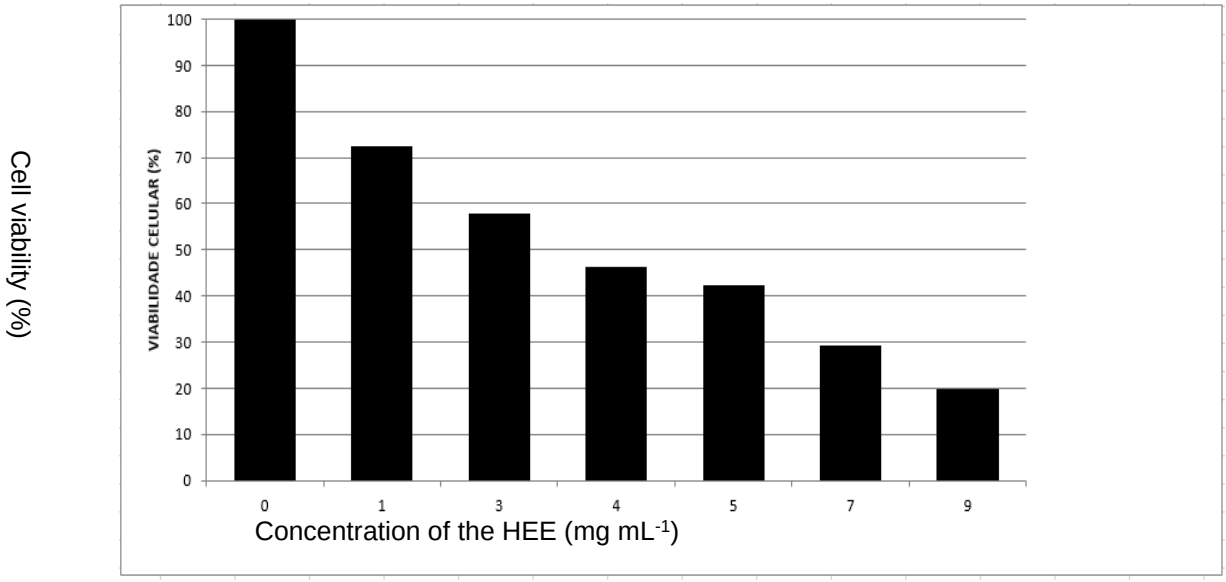
The percent inhibition (IP) of the samples (25 μg mL^{-1}) as well as the 50% efficient concentration (EC_{50}) were calculated using the 60 min data. The antioxidant activity index (AAI) was calculated by dividing the value of the final concentration of the sample by the EC_{50} at 60 min and classified as weak when AAI < 0.5, moderate when 1 > AAI > 0.5, strong when 2 > IAA > 1 and very strong when IAA > 2 (Scherer & Godoy, 2009). Statistical differences were determined by one-way ANOVA followed by Tukey's post-test ($\rho < 0.05$), with values with different letters indicating means with a significant difference. Source: Santos (2022).

Antiparasitic activity

The growth inhibition of *A. castellanii* trophozoites after exposure to the HEE of *G. americana* bark was concentration-dependent, that is, cell viability decreased when the HEE concentration increased, as shown in Figure 1.

For the different HEE concentrations (1, 3, 4, 5, 6, 7 and 9 mg mL^{-1}), cell viability ranged from 20% to 79% (from the highest to the lowest concentration, respectively) as see in Figure 1, where the positive control (amoeba + PYG medium) represented 100% of cell viability at absence of HEE. It can be seen that HEE at 3 mg mL^{-1} already presents an inhibition percentage of 42%. According to the non-linear regression equation used ($y = 23.514\ln(x) + 23.218$; $R^2 = 0.93$), the concentration at which the HEE is capable of inhibiting 50% of cell growth (IC_{50}) is 3.12 mg mL^{-1}.

Figure 1 - Amoeba Cell viability in different concentrations of the *G. americana* bark. Source: Santos, 2022.



Source: Authors.

The potential cell growth inhibition effect of the HEE was observed at 9 mg mL^{-1} as the cell viability was reduced by 80%. *G. americana* HEE is comprised of several classes of flavonoids, which the literature shows to be one of the secondary metabolite classes responsible for the biological activity of plants, including antiparasitic activity. Substances in this class of metabolites have the ability to complex with soluble and extracellular proteins, as well as the bacterial cell wall, disrupting these structures and killing cells of microorganisms (Colacite, 2015; Coutinho et al., 2009; Vunda et al., 2012).

Natural products are sources for the discovery of new bioactive compounds that may serve as therapeutic products for the treatment of diseases. The concentrations used in this assay can be considered relatively high, however, there are studies on the effects of plants on free-living amoebas with equivalent concentrations, as the study by POLAT et al. (2008), which used the methanolic extract of *Allium sativum* that showed efficacy at 3.9 mg mL^{-1} against the trophozoites of *A. castellanii*. Degerli et al. (2012) used methanolic extracts of *Origanum syriacum* and *Origanum laevigatum* at 32 mg mL^{-1} for assays against trophozoites of *A. castellanii*, which also showed efficacy against these forms of the parasite.

Therefore, it seems the *G. americana* L. bark HEE presents an unique potential for antiparasitic effect against the trophozoites of *A. castellani* after 24h. It should be noted the HEE incorporated into the O/W microemulsion was also tested. However, the microemulsion swelled when it was mixed with the medium placed on the plate wells, making the amoeba count unfeasible.

Microemulsion characterization

Droplet Size, pH determination and Polydispersity Index (PDI)

The analysis of the droplet size is carried out in order to verify if the formulations have a manometric size, which facilitates the incorporation of molecules, perfusion and dispersion in the target tissue. The polydispersity index reveals the homogeneity of the droplet population, characterizing a monomodal or polymodal size distribution (Soares, 2009). In the present study, it was observed the droplet sizes of the vehicle microemulsion (VME) and 10% HEE microemulsion (HEEME) were 20.29 nm and 18.33 nm, respectively, which are inside the limits established for microemulsions, from 10 to 30 nm in diameter, according to Pascoa (2012). The pH for the vehicle and 10% HEE microemulsions did not differ significantly ($p>0.05$) and showed a slightly acidic character (Table 3), being favorable for topical application considering that the skin pH is between 4.6 - 5.8 (Leonardi; Gaspar; Campos, 2001).

In addition, table 3 shows the polydispersity index was less than 0.5, which characterizes a homogenous distribution of droplets in both formulations (SILVA et al., 2014). RESENDE (2013) observed a similar behavior for microemulsions containing the essential oil of *Citrus sinensis* L., with PDI values from 0.1 to 0.32.

Table 3 - Physicochemical parameters of the vehicle microemulsion (VME) and 10% hydroethanolic extract microemulsion (HEEME) formulations.

FORMULATION	Droplet size (nm)	pH	PDI
VME	20.29	5.72 ± 0.24a	0.25
HEEME 10%	18.33	5.68 ± 0.29a	0.19

Values with different letters indicate means with a significant difference ($p<0.05$). Source: Santos (2022).

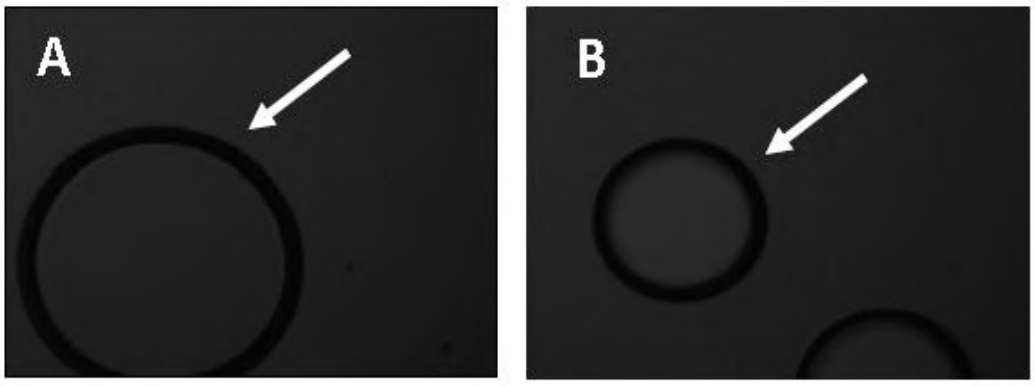
The development of delivery systems faces challenges because, after its administration in the body, it is necessary for the formulation to reach the target site and remain at the site of action for drug release in a controlled way, thus limiting adverse effects and ensuring biocompatibility (Gaumet et al., 2008). The elimination profile and tissue distribution of drug delivery systems are influenced by their size and surface characteristics (Gaumet et al., 2008), which makes it important to

know their physicochemical characteristics such as those produced in this study both for vehicle microemulsion and the one incorporating the HEE from the bark of *G. americana* L.

Polarized Light Microscopy (PLM)

According to the PLM test, the formulations of the vehicle microemulsion and 10% HEE in an oil/water microemulsion are characteristics of any microemulsion, which can be explained by the presence of dark fields (Carvalho et al., 2009) characterized by the presence of bubbles (Figure 2). In addition, there was no deviation or vibration of the polarized light and the formulations showed the same optical properties in all directions, characterizing themselves as an isotropic structure (Djekic; Primorac; Jockovic, 2011; Hyde, 2001).

Figure 2 - Photomicrographs, taken at 100x magnification, representative of isotropic behavior (dark field) obtained from formulation A) Assay 1: vehicle microemulsion (VME) at 6.3:0.7:3.0 (Tween 80 surfactant:oleic acid:water) and B) Assay 2: vehicle microemulsion + 10% HEE from *G. americana* bark (HEEME). The arrows indicate the presence of air bubbles to prove the dark field.



Source: Santos (2022).

4. Final Considerations

The hydroethanolic extract from the bark of *Genipa americana* L. has a potential antioxidant effect as well as an antiparasitic action against the protozoan *Acanthamoeba castellanii*, with an IC_{50} 3.12 mg mL^{-1}. The microemulsion was obtained as a carrier agent for the 10% extract to reduce the possible adverse effects and facilitate the absorption of the bioactive compounds present in it. The importance of obtaining new formulations using the HEE is justified as it may be an important source of substances for antiparasitic treatments.

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