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Antimicrobial activity of dermocosmetic formulations based on *Piptadenia gonoacantha*

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ABSTRACT

The search for herbal innovations with medicinal properties has intensified. As for this matter, the extracts of *Piptadenia gonoacantha*, present antimicrobial, anti-inflammatory and antinociceptive action, as well as absence of proven toxicity. The objective was to evaluate the antibacterial action of formulations containing extracts of *Piptadenia gonoacantha*. The extract was prepared and characterized by liquid chromatography coupled to mass spectrometry (UPLC-MS/MS). This was used for the development of cream, ointment, gel, balm and liquid soaps formulations, being submitted to evaluation, along with the extracts, for antibacterial activity. The characterization of the extract revealed the presence of rutin, ferulic acid, *p*-coumaric acid, quercitrin, canferol, apigenin and 6-hydroxycoumarin in their constitution. The formulations presented inhibition halos against strains of *S. aureus* and *S. epidermides*, with mean efficiency, relative to the positive control, of 47% to 99%. The best results were found for the *S. epidermides* strain with a mean of 91.5% efficiency in relation to the control. The presence of the metabolites observed in the characterization of the extract justifies the antibacterial action observed in its evaluation, as well as to the formulations. The formulations evaluated have promising antibacterial activity as natural therapeutic alternatives for the treatment of infectious processes.

Keywords: Anti-Bacterial Agents; Biological Products; Cosmetics

1 INTRODUCTION

The search for the natural, mainly nowadays, can be related to the difficulty of acquiring synthetic products or the high costs (BATISTA; VALENÇA, 2012). Other factors influencing the demand for traditional therapy are the growing tendency of the population to seek therapies that are less aggressive when compared to traditional medicines, or even because this increase in demand has driven research in the sector that ensures its effectiveness and safety (YUNES *et al.* 2001).

Another factor that may influence this search for the discovery and development of pharmacological effects of natural products, especially by plants, would be bacterial resistance (DEL FIOL *et al.* 2010). Bacterial drug resistance, available for clinical treatment, has become a worldwide public health problem (MCGOWAN, 2001). In addition, the financial cost of a failed therapy due to resistant microorganisms is very large, further burdening public health systems (DEL FIOL *et al.* 2010).

Several studies have been carried out in different countries to prove the efficacy of natural products of plant origin, in order to obtain alternative sources for the use of drugs obtained by synthesis, of which many microorganisms already have resistance (DJIPA *et al.* 2000; FERESIN *et al.* 2001; KHAN *et al.* 2001; AKINPELU; ONAKOYA, 2006; CHOPRA, 2007; SILVA *et al.* 2010; PADILHA *et al.* 2010; SANTOS *et al.* 2011; BONELLA *et al.* 2011; NATALLI *et al.* 2011; BELINELO *et al.* 2013).

Therefore, plants may contribute to the discovery of new antibiotics, with the presence of complex chemical structures that promote specific interactions and recognition by bacterial molecular targets differing from those already in the market (GUIMARÃES *et al.* 2010). Thus, this synergistic interaction between plant constituents differ the actions of the synthetic drugs that act individually (HEMAISWARYAA *et al.* 2008).

Phytotherapeutic medicinal products are characterized by being obtained from plant active raw materials, and their safety and efficacy are validated through technoscientific documentation in bibliographies and/or indexed publications, preclinical and clinical pharmacological and toxicological studies, without the use of active substance isolated (ANVISA, 2014).

Among thousands of species that make up Brazilian flora is *Piptadenia gonoacantha (Leguminosae-Mimosoideae)*, a tree species, common in the Atlantic Forest in the southern and southeastern regions of Brazil. Popularly it is known with the names of *"pau jacaré", "jacaré", "casco de jacaré",* among others (CARVALHO *et al.* 2014). The species Piptadenia gonoacantha possesses rapid leaf growth, widely used in reforestation for the recovery of degraded areas and restoration of preservation areas (LORENZI, 2008).

This species presents a good inhibitory potential for microorganism growth and anti-inflammatory action (CARVALHO *et al.* 2010). According to recent research, tests carried out with *Piptadenia gonoacantha* extract in different alcoholic degrees allowed to elucidate the relevant antibacterial activity against Staphylococcus aureus strain (ATCC 29213) and that such activity could be related to compounds already detected in the species, methyl, vitexin and isovitexin. The authors point out that vegetable species could serve as a future alternative for the elaboration of natural products with antibacterial activity (CARVALHO, 2014; CARVALHO *et al.* 2010), generating innovative healthcare products.

The development of extracts and natural products for the maintenance and restoration of skin integrity have been essential in the cosmetic area. It is noteworthy that substances from natural products rich in compounds such as flavonoids, for example, have low solubility in lipophilic systems (VISKUPICOVA *et*

al. 2009), presenting low penetration rate or skin absorption, which limits their bioavailability.

Therefore, in order to be more effective in the treatment of natural dermal products, they should be conveyed in cosmetic formulations, taking into account various factors such as the skin's diffusion coefficient, the type of formulation to be delivered. , among others (FLORENCE; ATTWOOD, 2011).

Since the addition of the extracts into the formulations, transforming them into the products, may lead to changes in biological activity, the search for experimental confirmation is recommended. Therefore, the main goal of this work was to perform more specific chromatographic elucidation studies of the standardized extracts of this species, as well as the development of dermocosmetic formulations with the extract and validation of the antibacterial effects of the formulations.

2 MATERIALS AND METHODS

2.1 Plant harvesting

Leaves of the species Piptadenia gonoacantha, popularly known as *"Pau Jacaré"*, were collected in the municipality of Viçosa, MG, Brazil, latitude 20º 45 '14 "S and longitude 42º 52' 55" W, altitude of 648 m. The harvest was carried out in September 2017, before flowering that occurs from January to August, in adult trees. The material was identified and authenticated by comparison with species present in the Botanic Garden of the Federal University of Viçosa-MG, where the specimen was deposited (exsiccate nº 35530).

2.2 Extraction process

The extracts of Piptadenia gonoacantha were obtained from their leaves, which were dried in a circulating air oven 40 ± 2 ºC for 96 hours and crushed in a knife mill. The powder obtained was standardized in 50 mesh (0.279 mm aperture) sieves where 97% of the powder passes through it and only 3% through the 60-mesh sieve (0.250 mm aperture).

To prepare the extract, the powder from the leaves of Piptadenia gonoacantha was used in the proportion 1:5 (100 g powder: 500 mL of 80% v/v ethanol/water solution with 0.3% citric acid), which is equivalent to the concentration of 20% dry extract (m/v). The extract was then subjected to the maceration process for 72 hours at a temperature of 25 ± 2 ºC, protected from light. Subsequent to vacuum filtration, the filtrate was reserved and the residue (pie) obtained in the funnel was extracted twice more. The filtrates were pooled at the end of the process in an amber bottle and lyophilized.

2.3 Obtaining the lyophilized extract

The obtained extract was subjected to a drying process by lyophilization under pressure of 10-1 mbar and temperature of -60 ºC. Subsequently, the lyophilized extract was evaluated for its phytochemical constitution and incorporated into the formulations.

2.4 Phytochemical characterization of the extract

Chromatographic separation was performed using C18 250 × 4.6 mm reverse phase column with pre-column of the same nature. The analysis was performed at room temperature (± 21 ºC), and the isocratic elution mode used orthophosphoric

acid (0.1%, m/m) as solvent A and acetonitrile as solvent B. The conditions of elution were: 80% A and 20% B (0-60 min), with the mobile phase flow of 0.8 mL min-1 (0-60 min), and the wavelength used for reading set on 210 nm. Liquid chromatography was coupled to mass spectrometry (UPLC-MS/MS), where 2000 ppm (plant mass/volume of solvent) were prepared, which were diluted to 500 ppm (plant mass/volume of solvent). The results obtained were expressed in relation to LODi (Detection Limit of the Instrument) and LOQi (Quantification Limit of the Instrument).

The following compounds were used as standard: gallic acid, chlorogenic acid, catechin, vanillic acid, caffeic acid, 6-hydroxycoumarin, p-coumaric acid, ferulic acid, rutin, 4-hydroxycoumarin, rosmarinic acid, quercitrin, myricetin, fisetin, resveratrol, trans-cinnamic acid, quercetin, luteolin, apigenin, kaempferol, 3,6-dihydroxyflavone, chrysin and galangin.

2.5 Preparing the formulations

Initially the lyophilized extract of P. gonoacantha was suspended in 80% (v/v) alcohol, in a ratio of 1:1 (1 g of lyophilized powder to 1 g of 80% alcohol). Subsequently, the obtained solution (50% - m/v) was incorporated in the pharmaceutical bases in the proportion of 20% in relation to the lyophilized extract.

Five bases of different pharmaceutical were manipulated, being characterized as ointment, gel, cream, balsam and liquid soap, according to the specifications of the National Formulary of the Brazilian Pharmacopoeia (ANVISA, 2012; CORRÊA, 2012).

Components such as 90% cetostearyl alcohol and 10% cetylstearyl sodium sulfate, almond oil, ethoxylated lanolin, liquid vaseline, solid vaseline, beeswax, preservatives and purified water were used for the preparation of the ointment base. The preparation technique follows what is recommended in the literature,

with the heating of the components until the wax melts and later on, agitation until the cooling (ANVISA, 2012).

The gel base was prepared with acrylic polymer, propylene glycol, ethylenediaminetetraacetic acid, methylparaben, imidazolidinyl urea, sodium hydroxide and purified water. The polymer was allowed to stand in water for 24 hours, until was fully solvated. After the other components were added, the pH was correct for the range to be between 6.5 and 7.5 which allows gel formation (CORRÊA, 2012; ANVISA, 2012).

On the other hand, the cream has components that are characterized by being in an oil phase: propylparaben, cetostearyl alcohol/ethoxylated sorbitan monostearate, butylhydroxy toluene and mineral oil; in an aqueous phase: methylparaben, propylene glycol, ethylenediaminetetraacetic acid and water were heated separately at 75 to 80 ºC. After heating, the aqueous phase was poured onto the oil phase with frequent stirring until cooling. The volatile component, cyclomethicone, was added after cooling (ANVISA, 2012).

The balsam base also had two distinct phases, so the oil phase components (butylhydroxytoluene, propylparaben, sunflower oil, copaiba oil, ethoxylated hydrogenated castor oil, and 90% cetostearyl alcohol and 10% sodium cetostearyl sulfate) were heated up until 70 ºC (± 5 ºC), separated from the aqueous phase components (pro-vitamin B5, methylparaben, caprylic capric acid triglycerides and demineralized water), which was also heated to 70 ºC (± 5 ºC) in a water bath. The components of the aqueous phase after heating were poured onto the components of the oil phase with constant stirring until cooling.

The liquid soap base was composed of sodium lauryl ether sulfate, coconut fatty acid diethanolamine, ethylenediamine tetraacetic acid, glycol distearate, cocoamidopropyl betaine, ethoxylated lanolin, glycerine, methylparaben,

propylparaben, citric acid and demineralised water, which were added in the sequence they are presented and homogenized. The viscosity of the formulation was adjusted at the final with the addition of sodium chloride (CORRÊA, 2012).

2.6 Evaluation of the antibacterial activity of the extract and the dermocosmetic formulations containing the extract of Piptadenia gonoacantha

The evaluation of the antibacterial activity of the extract of Piptadenia gonoacantha, as well as semi-solid formulations, ointment, gel and creams was carried out; and in liquid formulations, balsam and liquid soap containing the same extract.

2.7 Preparation of culture medium and bacteria

The analysis of the antibacterial activity of the species *P. gonoacantha* was carried out through the adaptation of the diffusion method in solid medium with perforation in agar. The microorganisms used in the assay were obtained from the Laboratory of Immunochemistry and Glycobiology of the Federal University of Viçosa - MG. Tests were performed with lyophilized extract in the following strains: *Staphylococcus aureus* (ATCC33591), *Staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC35984), *Escherichia coli* (ATCC 14948), *Bacillus cereus* (ATCC 14579) e *Proteus vulgaris* (ATCC 13315).

The Mueller-Hinton culture medium prepared according to the manufacturer's specifications was used in the tests. Bacterial cultures were maintained at 4 ºC in Mueller-Hinton. Before the tests, the strains were peeled into the medium and incubated at 36 ± 2 ºC for 24 hours. From recent cultures, bacterial suspensions were prepared in saline solution of 0.9% NaCl with turbidity equivalent to the McFarland Scale 0.5 (1.5×10^8 colony forming units (CFU)/mL) (NCCLS, 2003).

Absorbance control of these suspensions of microorganisms was carried out through spectrophotometer readings adjusted to the wavelength of 600 nm.

Subsequently, 500 µL of this suspension was mixed in the liquid sterile Mueller-Hinton medium (12.5 mL) at 37 ºC, and then poured into sterile Petri dishes (90 mm diameter). The wells were made using a vacuum pump coupled to a sterile tip previously adapted for this purpose. In each plate wells were drilled for application to different concentrations of the extract (10%, 20%, 30%, 40% and 50%), as well for application of the positive control (ampicillin 50 mg/mL) and for the negative control (80% ethanol).

20 µL of the extract, its dilutions, the positive (ampicillin 50 mg/mL) and negative (alcohol 80%) controls were deposited in the wells corresponding to each one. After incubation for 24 hours in an oven at 37 ºC, the diameters of the inhibition halos were measured in mm, and the results were organized and described.

For the evaluation of the antibacterial activity of the formulations, wells were drilled for the product to be applied (base + extract), one for the positive control (ampicillin 50 mg/mL), one for the extract in the concentration used in the formulation (20% diluted in alcohol), one for alcohol 80% and another for negative control (base of the formulations without the extract of Piptadenia gonoacantha). The deposited amounts for controls and products were 20 µL. After incubation for 24 hours in an oven at 37 ºC, the diameter of the inhibition halo was measured in mm, and the results were organized and described.

2.8 Statistical analysis

The data was submitted to analysis of variance and the comparison of means by the Tukey test at 5% probability using the STATA version 13 software. Descriptive

analysis was performed through the mean and standard deviation for the variables and the presentation of the data in tables.

3 RESULTS AND DISCUSSION

3.1 Preparation of extracts

The extract of Piptadenia gonoacantha in 80% alcohol was obtained after the maceration process of the dry plant powder, and the use of citric acid (0.3%) during the extraction process is justified by its potential to be a flavonoid preservative according to Novello (2011).

Every procedure has been thoroughly evaluated and validated for best results against an antibacterial activity of extracts in previous studies by our team (CARVALHO *et al.* 2014) Thus, the findings of this study indicate the best conditions for obtaining the standardized extract of Piptadenia gonoacantha by the extraction method used. This is a significant contribution to the development of a new phytopharmaceutical intermediate product from this herbal drug.

The yield obtained was 24.17% of lyophilized extract, according to the calculations presented below:

$$Re = \left(\frac{37.70}{156}\right) x100$$

$$Re = 24.17\%$$

3.2 Phytochemical Characterization

The presence of 6-hydroxycoumarin, p-coumaric acid, feluric acid, rutin, quercitrin, apigenin and kaempferol, at varying concentrations were identified (Table 1).

These compounds have been reported as the main compounds linked with the pharmacological actions studied in this work

Table 1 – Metabolites evaluated by liquid chromatography coupled to mass spectrometry (UPLC-MS/MS) of the hydroalcoholic extract 80% of *P. gonoacantha* and their reported pharmacological activities in literature

Compound	Results (ng/mL)	Pharmacological activities
Rutin	33.4	Anti-inflammatory (Coutinho *et al.* 2009; Ganeshpurkar & Saluja, 2017; Gullón *et al.* 2017), antidiabetic and antimicrobial, anticancer, antiallergic (Gullón *et al.* 2017).
Feluric acid	11.6	Anti-inflammatory, anticancinogenic, hepatoprotective, neuroprotective (Srinivasan *et al.* 2007; Ghosh *et al.* 2017), antimicrobial (Borges *et al.* 2013; Wang *et al.* 2017), antidiabetic and antioxidant (Srinivasan *et al.* 2007; Ghosh *et al.* 2017; Nankar *et al.* 2017).
p-coumaric acid	10.5	Antibacterial, antioxidant (Stojković *et al.* 2013; Kim *et al.* 2017), anti-inflammatory, anti-ulcerogenic and anti-mutagen (Stojković *et al.*, 2013).
Kaempferol	7.6	Prevention and treatment of inflammatory diseases, pain, diabetes, infections (Calderón-Montaño, 2011), neuroprotective inhibits oxidation and anti-inflammatory (Wu *et al.* 2017).
Quercitrin	7.4	Antioxidant (Babujanarthanam *et al.* 2011; Cincin *et al.* 2014), antidiabetic (Babujanarthanam *et al.* 2011, Babu *et al.* 2013) and anticarcinogenic (Cincin *et al.* 2014).
Apigenin	2.2	Antidiabetic (Babu *et al.*, 2013), neuroprotective (Nabavi *et al.* 2017), antioxidant (Nabavi *et al.* 2017; Salmani *et al.* 2017; Telange *et al.*, 2017) and anticarcinogenic (Salmani *et al.* 2017; Madunić, 2018).
6-hydroxycoumarin	1.9	Cytotoxic to carcinogens (Montagner, 2007; Rehman *et al.* 2014).

Rutin, a flavonoid subclass of flavonols (RODRIGUES DA SILVA *et al.* 2015), was the compound found in the highest concentration, 33.4 mg/mL extract. The presence of other flavonoids such as quercitrin (74 ng/mL) and kaempferol (7.6 ng/mL) of the flavonol subclass may also be observed; besides the presence of apigenin (2.2 ng/mL) of the flavone subclass.

The flavonoids present in plants perform several functions, such as protection against solar radiation, insects and microorganisms, besides having antioxidant activity (COUTINHO *et al.* 2009).

Therefore, the presence of the flavonoids rutin, quercitrin, apigenin and kaempferol, can justify the antimicrobial results obtained in biological tests in vitro.

According to Havsteen (2002), the use of flavonoids against bacterial, protozoan, and fungal infections has two purposes: (1) to kill the bacterial or fungal cells and (2) to counteract the spread and the effects of the bacterial toxins (HARBORNE *et al.* 1976; MCCLORE, 1975; LOPES *et al.* 1998). Many, but not all, of the bacterial strains commonly encountered by humans are killed by flavonoids (BAGAEV, 1978; EL-GAMMAL; MANSOUR, 1986). However, the mechanism by which this is accomplished is not known yet. Since eicosanoids do not appear to be formed by bacteria, the primary targets of the flavonoids, the PG COX and the related enzyme lipoxygenase, do not come in question since only eukaryotic cells, including plants, possess such enzymes. Neither does another important target, the cAMP PDE, since bacteria, like other prokaryotic cells, do not possess this enzyme. However, they do contain metalloenzymes, the heavy metal atoms that form strong ligand complexes with flavonoids, e.g., phosphatases. Therefore, the bactericidal effect of the flavonoids may well be the result of a metabolic perturbation. Ion channels, which are components of both bacterial and animal cells, are especially sensitive points of inhibition and likely targets of flavonoids. In animal cells, these channels are regulated by phosphorylation/dephosphorylation reactions. Fungi, which often accompany bacterial infections, may be killed by flavonoids due to any of the two mechanisms mentioned above. All infectants, including viruses, may be eliminated through the immunostimulatory effect of flavonoid treatment (OHNISHI; BANNAI, 1993; CONTI *et al.* 1998).

Apart from the active role that the flavonoids play in the destruction of infectants, they fortify loose connective tissues by inhibiting some of the enzymes that can hydrolyze their proteoglycan and protein meshwork. This mesh sterically hinders the diffusion of infectants through the tissue. One example is the inhibition of hyaluronidase by flavonoids. Thus, the latter contribute to the immobilization and encapsulation of the infectants.

Among the identified flavonoids is the rutin, that has been reported by many researchers to be found in plant species of great pharmacological interest for

human health, besides possessing many biological properties, such as antiallergic, anti-inflammatory, antitumor, antibacterial, antiplatelet properties, antispasmodic, antiviral, antiulcerogenic, antidiarrheal, vasodilator, cytoprotective, antihypertensive, antimutagenic, protection of the hepatocellular lesion and antioxidant activity by the elimination of reactive oxygen species, such as hydroxyl radical ($OH^{\bullet}$), superoxide radical anion ($O_2^{\bullet -}$) and peroxide radical ($R\text{-}O\text{-}O^{\bullet}$) (JANBAZ *et al.* 2002; CALABRÒ *et al.* 2005; CAILLET, *et al.* 2007; JIANG *et al.* 2007; YANG *et al.* 2008; DOMITROVIC *et al.* 2012; MAHMOUD, 2012; OLIVEIRA, 2015).

Quercitrin has been used as a bacterial agent (CINCIN *et al.* 2014). However, studies have shown that it does not interfere with bacterial growth but inhibits the activity of the enzyme Sortase A (LIU *et al.* 2015). Sortase A (SrtA) is known as the "home organization" of Gram-positive bacteria and is often involved in the pathogenesis of these bacteria. This enzyme is responsible for adherence to the tissue, and when it is inactivated, the bacterium has the reduced capacity to infect the host (MAZMANIAN *et al.* 2000).

This information reinforces the idea that the set of substances present in plants can act in a synergistic way increasing, in these case, the antibacterial action and because it is not an isolated substance, makes it more difficult the process of adaptation of the bacterium to a harmful compound.

Kaempferol is also classified as a flavonoid and therefore has antioxidant activity as rutin and quercetin (DORNAS *et al.* 2007). The kaempferol also has antibacterial activity against the bacteria Stafilococcos aureus, as observed by Resende *et al.* (2015). The authors observed that the antibacterial effect of kaempferol was superior to other flavonoids in the study, such as quercetin, and that this fact could be related to the lipophilic balance of the molecule (RESENDE *et al.* 2015).

Therefore, the different flavonoid molecules found in Piptadenia gonoacantha could use different mechanisms in the antibacterial action, also in comparison to conventional drugs, which would be a further justification for their use in infections, and even in research against resistant bacteria.

The coumarin derivatives, such as 6-hydroxycoumarin present in Piptadenia gonoacantha, present pharmacological activities as anti-inflammatory (LEITE et al. 1993) and bactericidal (SILVA *et al.*, 2016), and are also used as anticoagulants (KOSUGE *et al.* 1985; KO *et al.* 1989; CHEN *et al.* 1995) and vasorelaxants (LEMMICH *et al.* 1983).

The hydroxycinnamic acids, such as ferulic and p-coumaric acids, are phenolic compounds that have antioxidant properties and assist in inflammatory processes (HRAZDINA *et al.* 1970; OLIVEIRA; BASTOS, 2011). Apigenin, as well as quercetin, has an anti-inflammatory action, that works reducing the production of nitric acid or inhibiting the enzyme cyclooxygenase (COUTINHO *et al.* 2009).

The presence of flavonoid compounds that have antimicrobial activity in the analyzed extract confirm the antimicrobial property found in other analyzes of antibacterial activity. In addition to this activity, the presence of compounds that have anti-inflammatory activity, rutin, 6-hydroxycoumarin and apigenin, may be an indicative that the formulation obtained has an anti-inflammatory effect.

The immune response has a role in defense against microorganism, and in the skin, among other processes, there is the release by keratinocytes of cytokines that recruit inflammatory cells and lymphocytes (MACHADO *et al.* 2004). Therefore, the presence of compounds with anti-inflammatory activity in the extract of Piptadenia gonoacantha, may contribute to the process of reestablishing an infectious process.

The presence of kaempferol and other flavonoids, which have antioxidant activity recognized, could add not only to the treatment of infections or skin lesions, but also be an alternative to the cosmetic market as anti-aging assets. Therefore, such evidences are important conditions for carrying out in vivo assays to prove such effects.

3.3 Evaluation of the antibacterial activity of the extract and the formulations containing the extract of Piptadenia gonoacantha

The lyophilized extract of *P. gonoacantha* presented inhibition halos against *Staphylococcus aureus* (ATCC33591), *Staphylococcus aureus* (ATCC29213) and *Staphylococcus epidermidis* (ATCC35984). Table 2 shows the values of the inhibition halos at different concentrations of the lyophilized extract. In relation to *the S. aureus* strain (ATCC33591), there was no statistical difference between the sizes of the inhibition halos in concentrations of 10% to 40%. For the *S. aureus* strain (ATCC29213), there was no difference between the halos of 20% to 50%, and for this strain, the values found were higher than the positive control. In the *S. epidermidis* strain (ATCC35984), inhibition halo values also had no statistical difference in concentrations of 20% to 50%.

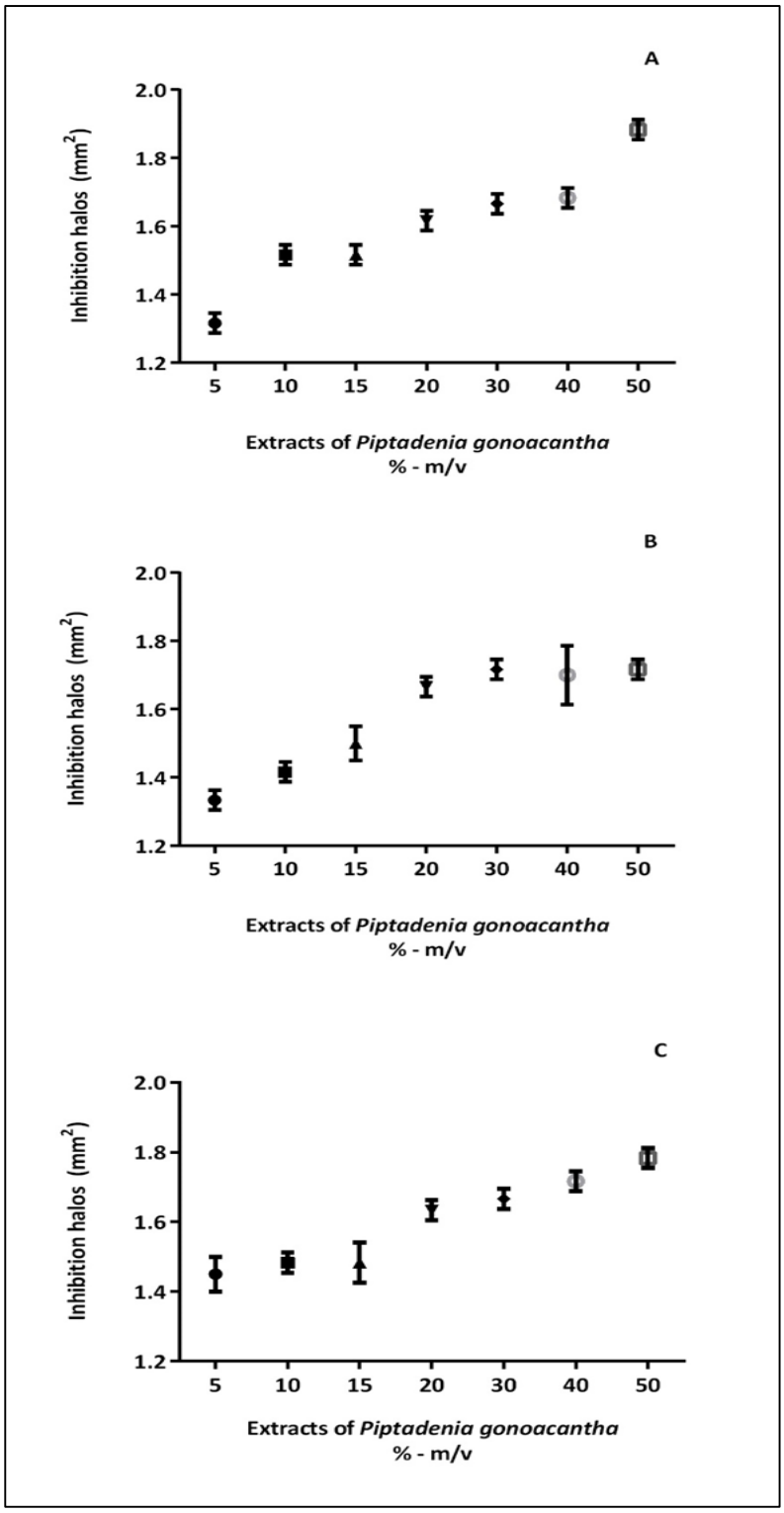
Table 2 - Halo of bacterial inhibition for different concentrations of the extract *Piptadenia gonoacantha*

Concentrations of *P. gonoacantha* extract	***S. aureus* (ATCC33591)**	***S. aureus* (ATCC29213)**	***S. epidermidis* (ATCC35984)**
5%	1.32 ± 0.03^{E}	1.32 ± 0.06^{C}	1.43 ± 0.05^{D}
10%	1.53 ± 0.03^{D}	1.43 ± 0.03^{BC}	1.48 ± 0.03^{D}
15%	1.52 ± 0.03^{D}	1.50 ± 0.05^{BC}	1.45 ± 0.06^{D}
20%	1.62 ± 0.03^{CD}	1.67 ± 0.03^{A}	1.62 ± 0.03^{C}
30%	1.67 ± 0.03^{CD}	1.72 ± 0.03^{A}	1.67 ± 0.03^{BC}
40%	1.68 ± 0.03^{CD}	1.70 ± 0.09^{A}	1.72 ± 0.03^{BC}
50%	1.88 ± 0.03^{B}	1.72 ± 0.03^{A}	1.78 ± 0.03^{BC}
C+*	2.32 ± 0.03^{A}	1.43 ± 0.02^{BC}	2.13 ± 0.06^{A}

*C+: Positive Control (ampicillin 50 mg/mL) **Means followed by equal letters in the column, for each of the strains alone, did not differ statistically from each other by the Tukey's test (P <0.05)

In Figure 1 it is possible to observe the relationship between dose and response of the different dilutions of the *P. gonoacantha* extract against *Staphylococcus aureus* and *Staphylococcus epidermidis* strains.

Figure 1 - Dose response relationship of different dilutions of the *Piptadenia gonoacantha* extract against strains A - *Staphylococcus aureus* (ATCC33591), B - *Staphylococcus aureus* (ATCC29213) and C - *Staphylococcus epidermidis* (ATCC35984). R – Replicates



The formulations of ointment, gel, cream, balsam and liquid soap prepared with Piptadenia gonoacantha extract in a concentration of 20% when compared to the lyophilized extract showed antibacterial activity against strains of *S. aureus* (ATCC33591), *S. aureus* (ATCC29213) and *S. epidermidis* (ATCC35984) (Table 3). The 20% concentration was the one of choice because it did not differ statistically from the higher concentrations that were evaluated, besides the fact that it was a concentration that did not interfere pharmacotechnically in the production of the formulations.

The formulations containing the extract of *P. gonoacantha* presented inhibition halos against the three strains analyzed *S. aureus* (ATCC33591), *S. aureus* (ATCC292130) and *S. epidermidis* (35984) (Figure 2), with average relative efficiency, in relation to the positive control (ampicillin 50 mg/mL), from 47% to 99% (Table 3).

Figure 2 - Inhibition halos of the formulations containing the extract of *Piptadenia gonoacantha* 20% and of the positive control in the strains of *S. aureus* and *S. epidermides*. Bal - Balsam; Cre – Cream; Gel – Gel; Oin – Ointment; Liq - Liquid soap; Amp – ampicillin

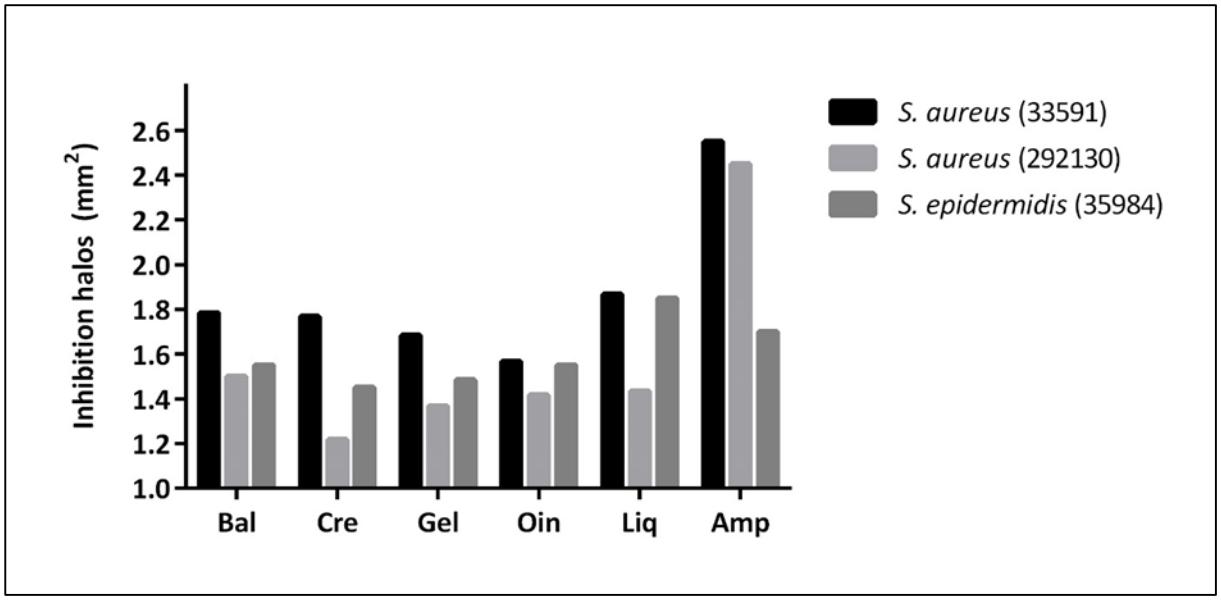


Table 3 - Efficacy relative to the positive control of ointment, gel, cream and balsam formulations containing 20% *P. gonoacantha* extract (PG)

	Inhibition Halo / cm		
	***S. aureus* (33591)**	***S. aureus* (292130)**	***S. epidermidis* (35984)**
Ointment			
20% PG extract in alcohol 80%	2.10 ± 0.06	1.40 ± 0.05	1.75 ± 0.04
Positive control (ampicillin 50 mg/mL)	2.75 ± 0.06	2.60 ± 0.06	1.68 ± 0.02
Ointment of PG 20%	1.57 ± 0.09	1.22 ± 0.02	1.45 ± 0.07
% effectiveness of the formulation*	57%	47%	86%
Negative control (alcohol 80%)	0	0	0
Ointment base (without active)	0	0	0
Gel			
20% PG extract in alcohol 80%	1.96 ± 0.05	1.40 ± 0.05	1.77 ± 0.03
Positive control (ampicillin 50 mg/mL)	2.75 ± 0.05	2.65 ± 0.04	1.65 ± 0.03
Gel of PG 20 %	1.68 ± 0.08	1.37 ± 0.09**	1.48 ± 0.02
% effectiveness of the formulation*	61%	52%	90%
Negative control (alcohol 80%)	0	0	0
Gel base (without active)	0	0	0
Cream			
20% PG extract in alcohol 80%	2.15 ± 0.06	1.40 ± 0.05	1.80 ± 0.04
Positive control (ampicillin 50 mg/mL)	2.80 ± 0.04	2.60 ± 0.06	1.66 ± 0.02
Cream of PG 20 %	1.77 ± 0.09	1.42 ± 0.08**	1.65 ± 0.07
% effectiveness of the formulation*	63%	55%	99%
Negative control (alcohol 80%)	0	0	0
Cream base (without active)	0	0	0
Balsam			
20% PG extract in alcohol 80%	2.10 ± 0.06	1.36 ± 0.05	1.75 ± 0.04
Positive control (ampicillin 50 mg/mL)	2.80 ± 0.05	2.50 ± 0.06	1.70 ± 0.02
Balsam of PG 20 %	1.72 ± 0.06	1.55 ± 0.07	1.55 ± 0.07

Continuatioin...

Conclusion			
Balsam			
% effectiveness of the formulation*	61%	62%	91%
Negative control (alcohol 80%)	0	0	0
Balsam base (without active)	0	0	0
Liquid soap			
20% PG extract in alcohol 80%	2.10 ± 0.06	1.40 ± 0.05	1.75 ± 0.04
Positive control (ampicillin 50 mg/mL)	2.75 ± 0.06	2.60 ± 0.06	1.68 ± 0.05
Liquid soap de PG 20 %	1.87 ± 0.09	1.43 ± 0.09	1.85 ± 0.07
% effectiveness of the formulation*	-	-	-
Negative control (alcohol 80%)	0	0	0
Liquid soap base (without active)	1.13 ± 0.09	0.88 ± 0.02	1.22 ± 0.03

* Relation between the inhibition halo of the formulation and the inhibition halo of the positive control.
** Values that did not differ statistically from the inhibition halo of the 20% Piptadenia gonoacantha extract.

In the case of the *S. aureus* strain (ATCC33591) the mean was 60.5%, except for the liquid soap-type formulation which showed inhibition halo with the basic composition of the liquid soap. However, the addition of the extract did not substantially increase the inhibition of bacterial growth.

For the *S. aureus* strain (292130), a mean of 54% efficacy of the pharmaceutical formulations was observed, except for *S. aureus* strain (ATCC33591), where the liquid soap itself was shown active against the microorganism leading to a slight increase of this effect with the addition of the extract of *Piptadenia gonoacantha*.

The antibacterial effect of the formulations on *S. epidermidis* (35984) were more efficient when compared to the *S. aureus* strains, obtaining an average of 91.5% in relation to the positive control. In the case of *S. epidermides*, the basic pharmaceutical formulation of liquid soap presented inhibition halo and as for the other bacteria, the addition of the extract also led to a discrete increase of the inhibition halo.

The formulations in the ointment pharmaceutical form had the lowest percentages of effectiveness in relation to the positive control when compared to gel, cream and balsam formulations. Possibly this occurred due to its increased lipophilic characteristic that hinders the transfer of the drug to the hydrophilic agar medium (LÜLLMAN *et al.* 2010).

For the drug to perform its therapeutic action it must leave the pharmaceutical preparation and act on the skin. For a better yield of the drugs to the skin, they should differ as to the chemical characteristics in relation to the base, i.e., a hydrophilic drug and a lipophilic base, the tendency is that the drug is more available (HEIZN *et al.* 2010). The ointment was expected to perform better, but the characteristics of the in vitro experiment are chemically differentiated relative to the skin profile. Therefore, the ointment is expected to perform better in vivo tests. Another important feature of ointment that also influences in vivo activity is the fact that its lipophilic nature provides an occlusion in the dermis, increasing hydration at the application site, allowing a better penetration of the drug into the skin.

The other formulations maintained inhibition halos with values close to the halos of the *P. gonoacantha* extract at 20% concentration. In the case of the *S. aureus* strain (292130) the gel and cream formulation showed statistically similar inhibition halos.

The gel formulation, despite having the characteristic ability to release the drug more easily when compared to ointment and cream (LOURENÇO, 2013), did not show this efficiency in the analyzes performed. Probably, this is due to the fact that the flavonoids have hydrophilic characteristics as well as the gel, therefore, makes it difficult to transfer the drug to the dermis. In these cases, a good resolution is making changes in the constitution of the formulation by adding inputs that would increase the permeability of the drug.

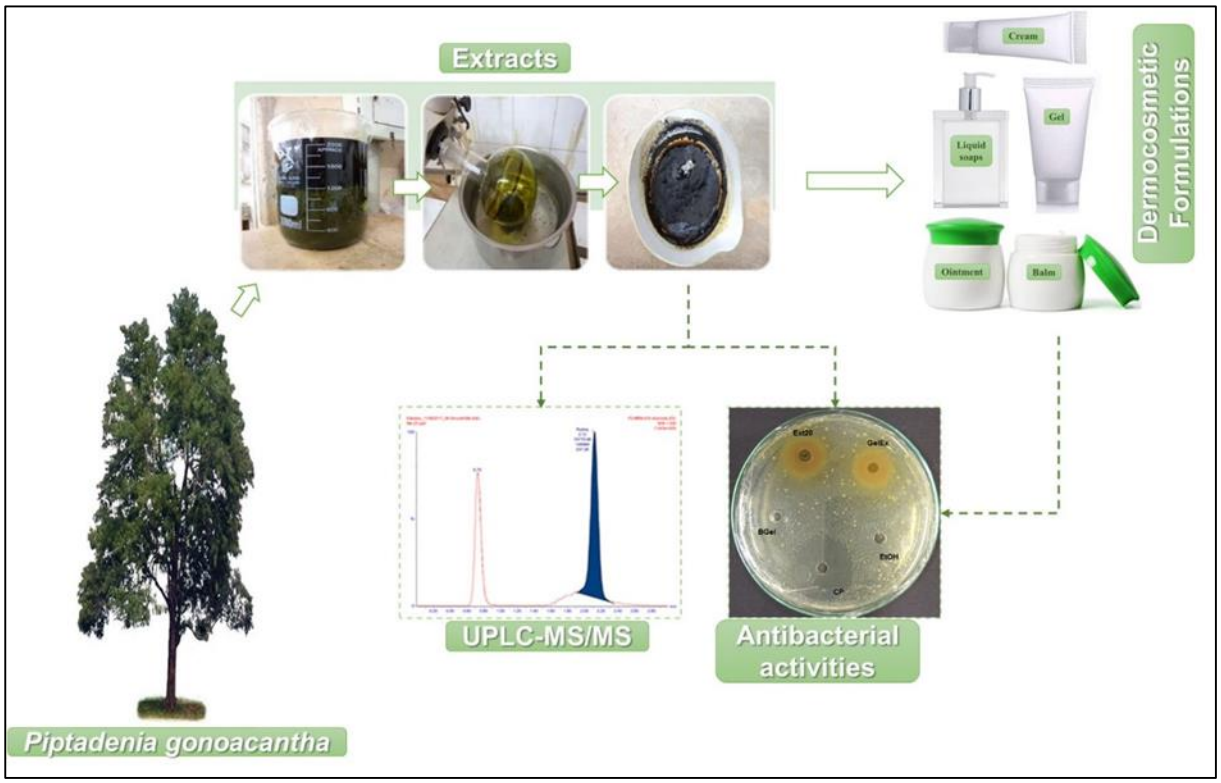
Some ways to improve the permeability of the drug in the skin would be to increase hydration, in this case the formulations containing more fatty substances and which are more occlusive (WILLIAMS; BARRY, 2004) such as cream, ointment and balsam, would allow a better permeation of the drug. Another way would be the addition of alcohols that can act favoring the solubility of the active ingredient, altering the solubility properties of the cutaneous tissue, among others. Polyethylene glycol would be an example of an alcohol that can be added to the formulations alone or associated, in which case its association with oleic acid has a synergistic effect on the permeability (MARTINS; VEIGA, 2002; WILLIAMS; BARRY, 2004; LANE, 2013).

The formulation of the cream type was the one that presented the best results, possibly due to the characteristics of having fatty substances in its composition, which allow the maintenance of moisture in the corneum extract that helps the penetration of the drug. There is also the possibility of the association of the surfactant, which allows to maintain the hydro-lipophilic balance of the formulation, solubilize lipid substances of the stratum corneum favoring permeability (WILLIAMS; BARRY, 2004).

The effects of the formulations against the *S. epidermides* strain, especially in relation to the gel, cream and balsam formulations, were highlighted in relation to the positive control, yielding a percentage of effectiveness higher than 90%.

The in vitro assay confirmed that the formulations produced with 20% of the alcoholic extract of *P. gonoacantha* have similar antibacterial activity to the hydroalcoholic extract solubilized in the same concentration (Figure 3). However, due to the particularities of each vehicle there were some differences in values between them. Each vehicle provides the drug according to the chemical characteristics of both and the medium to which they were submitted.

Figure 3 – Antimicrobial activity of dermocosmetic formulations based on Piptadenia gonoacantha



The fact that the results were positive is already an indicative that these formulations are likely to be used in the future as a medicine for skin infections. It also allows a targeting for a study viewing an optimization of drug delivery, thus improving the therapeutic activity of the same.

The drug obtained from an extract of a plant brings the possibility of a treatment with a product that is more likely safer and promote fewer undesirable reactions. Another possibility, which should be better evaluated, would be to assay its action in bacterial strains resistant to other antibiotics.

The extract of *Piptadenia gonoacantha* presents in its chemical constitution phenolic compounds that are known to have antibacterial action, such as rutin, quercetin and 6-hydroxycoumarin. The formulations with *P. gonoacantha* extract also showed antibacterial activity similar to the extract and substantial equivalence to controls.

The fact that the active input is withdrawn from nature, of a large tree, widely distributed in the Atlantic forest, of easy proliferation, reduces the costs of the final formulation. Thus, it can be said that besides the advantage of being a product that can be obtained from raw material of vegetal origin, also has the benefit of a low cost.

The production of the ethanolic extract of *Piptadenia gonoacantha* requires a low-cost structure, which added to the final products, allows the development of an accessible innovation. In addition, this study serves as a stimulus to entrepreneurship and innovation in academia, and it is possible to promote partnerships between the public sector and private companies, favoring the country's economic development, as well as giving a return to society on public investments in research.

Further investigations with Piptadenia gonoacantha species should look for products with better action and clinical safety. It would also be interesting to investigate the mode of action of the extracts against test bacteria and resistant clinical strains.

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