ORIGINAL ARTICLE

Development of an antifungal liquid soap for gynecological use from extract of the leaves of *Astronium urundeuva* (aroeira-do-sertão)

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ABSTRACT

Aroeira-do-sertão [*Astronium urundeuva* (M.Allemão) Engl.] is a plant with medicinal properties useful in the management of vulvovaginal candidiasis (VVC). Thus, the objective was to develop gynecological liquid soap with antifungal action from fluid extract of aroeira-do-sertão (EFA). The leaves of the adult tree were collected and processed, and the plant drug was characterized. To obtain the EFA, maceration was used followed by percolation with a hydroalcoholic solution (60%), with phytochemical identification and measurement of active markers, polyphenols (TP) and total tannins (TT). The antifungal action of EFA against *Candida* strains was also evaluated using the agar diffusion technique and the minimum lethal concentration (MLC) was determined. The liquid soap from aroeira-do-sertão (SLS) was evaluated for its quality and antifungal action, being compared to the results of a commercial intimate soap containing aroeira-da-praia extract (*Schinus terebinthifolia* Raddi – PLS). An EFA of adequate quality was obtained, with a TP content equal to 189.08 (±0.0306) mg EAG/g of EFA, of which only 9.43% corresponds to TT. EFA proved to be effective against *C. albicans* ATCC 10231, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 13803, with MLC of 0.5% (w/v). SLS with 2.5% w/v EFA showed appropriate quality and antifungal effect similar to that found for EFA, while PLS exhibited lower antifungal activity due to its composition.Therefore, the formulated intimate liquid soap demonstrated relevant antifungal activity, confirming its potential as an alternative for the management of vulvovaginal candidiasis..
Keywords: candidiasis; polyphenols; pharmaceutical preparations; pharmaceutical technology; *Myracrodruon urundeuva*..

INTRODUCTION

Aroeira-do-sertão is the common name for a botanical species belonging to the Anacardiaceae family, whose scientific name is *Astronium urundeuva* (M.Allmeão) Engl. [sin. *Myracrodruon urundeuva* M.Allemão] (Domingos and Silva 2020).

Chemical studies have revealed the presence of numerous phenolic compounds in the leaves of *A. urundeuva*, such as tannins (gallic acid) and flavonoids (quercetin, agatisflavone and aromadendrinol) (Bandeira 2002; Galvão 2018; Sousa et al. 2022).

These polyphenols are part of the phytotherapeutic complex responsible for the therapeutic activities of its plant derivatives, such as the *in vitro* antifungal action of aroeira-do-sertão extracts against species of the genus *Candida* (Oliveira et al. 2017; Hsu et al. 2021).

Thus, the aforementioned medicinal plant has ethnopharmacological and scientific data that have aroused interest in its pharmaceutical, cosmetic and hygiene product applications, and has already been included in the Farmácias Vivas Program through presentations such as elixir and vaginal cream (Bandeira 1993, 2002; Galvão 2013; Galvão 2018).

This therapeutic use is particularly relevant in the development of antifungal products for the treatment, hygiene and prophylaxis of infectious diseases, such as vulvovaginal candidiasis (VVC). VVC is a disease caused mainly by *C. albicans* and has a high prevalence, which results impacts on patients' health, sexuality and self-esteem (Sustr et

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al. 2020; Cruz et al. 2022).

From this perspective, the present study aimed to develop a liquid soap for gynecological use with antifungal action against yeasts of the genus *Candida* using an extract characterized from *A. urundeuva* leaves.

MATERIAL AND METHODS

Plant drug processing and characterization

The leaves of adult specimens of aroeira-do-sertão were collected at the Family Development Center on Federal University of Ceará, Fortaleza, Brazil (3°44'33.0"S; 38°34'37.6) at 9 am in October 2023. Exsicata was registered under number #33006 in the Prisco Bezerra Herbarium of the UFC Biology Department, being identified by botanist Igor Renan Bonfim de Souza, and the genetic heritage was registered in National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under access number A9B8196.

The plant material was scraped, dried in an oven with air circulation at 40 °C for 24 h and crushed in knife mills with a 2 mm mesh opening, and the particle size distribution was evaluated with a sequence of sieves opening from 2 to 0.18 mm (Galvão 2013).

In the vegetable drug obtained, total ash was determined by incineration in a muffle furnace with a temperature gradient, in addition to loss by desiccation using the gravimetric method, both in triplicate. The desiccation loss test was also carried out on fresh and dried leaves (Anvisa 2024a).

To determine the phytochemical profile, the pulverized plant drug was subjected to hot extractions in aqueous, aqueous with 2% sulfuric acid, 70% hydroalcoholic and chloroformic media. To the extracts obtained at a concentration of 12% of the plant drug, specific reagents were added that resulted in a change in color, formation of precipitate or foam (Matos 2009).

Production and quality control of plant extract

To prepare the fluid extract of aroeira-do-sertão (EFA), the vegetable drug was moistened with the mixture ethanol:water:glycerol (60:40:5, v/v) and left to macerate for 6 h. Then, 128 ml of the percolate was collected, which was stored in a refrigerator at 6,0 °C. Finally, the ethanol:water solution (60:40, v/v) was added to the vegetable drug that remained in the percolator and the percolate obtained was concentrated in a water bath until a total of 160 ml of EFA was obtained (Galvão 2018; Anvisa 2024a).

The characterization of EFA was carried out through the evaluation of organoleptic characters, pH by potentiometry, phytochemical prospecting and content of active markers (Bastos et al. 2020; Anvisa 2024).

Phytochemical prospecting for EFA was carried out according to the technique described for the plant drug, except for the use of extract solutions diluted at a concentration of 6%, considering the extract itself as a solute (Matos 2009).

Regarding specifically the qualitative identification of flavonoids, the fluid extract was mixed with silica gel until obtaining a dry extract, which was added to the stationary phase of silica 60 for the column (250 x 15 mm) and eluted sequentially by hexane, dichloromethane, ethyl acetate and methanol, until a change in color of the liquid collected at the end of the chromatographic separation was observed (Bandeira 2002). The collected organic phases were evaporated to dryness on a heating plate, reconstituted with 70% ethanol and subjected to the cyanidin reaction, in which the color change to reddish tones confirms the presence of flavonoids (Matos 2009).

The determination of the content of total polyphenols (TP) and total tannins (TT), active markers of the fluid extract of aroeira, was carried out through an oxidation-reduction reaction with Folin-Denis reagent (400 µl for every 10 ml of solution) in alkaline medium (29% sodium carbonate solution), with the blue complex formed being read on the spectrophotometer at 718 nm (Galvão 2018; Anvisa 2024b).

Gallic acid was used to perform the calibration curve (1, 2, 3, 4, 5, and 6 µg/mL) and to evaluate the repeatability (sextuplicate) of concentration 3 µg/ml of the method and, therefore, the results for analyte content in EFA were expressed in gallic acid equivalents (GAE) (Anvisa 2017).

Antifungal activity of the plant extract

The antifungal activity of EFA against fungi of the genus *Candida* was evaluated using the agar diffusion technique, whose methodology was adapted from those recommended by Anvisa (2024a) and BrCAST (2021), considering the minimum lethal concentration (MLC).

Fluconazole was used as a reference antifungal at concentrations of 0.5, 0.25, and 0.125% (w/v), with water as the solvent, while EFA was diluted in 1% sodium lauryl sulfate (SLS) for the preparation of a solution at a concentration of 2% (w/v) and, from there, serial dilutions were made to obtain concentrations of 1 and 0.5% (w/v) in water (Freitas et al. 2014).

To prepare the microbial inoculum, standard strains *C. albicans* ATCC 10231, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 13803 in a vegetative state were sown on a Sabouraud-dextrose agar (ASB) slant and

incubated at 35,0 ± 2 °C for 24 h for activation, and then dispersed in Sabouraud broth until a microbial suspension with equivalent turbidity was obtained to tube 4 on the *McFarland scale* (12 x 10^6 CFU/ml), measured by reading an absorbance value equal to 0.68 at 625 nm on a photocolorimeter (BrCAST 2021).

Finally, the standardized inoculum was diluted in ASB in a 2:100 ratio, resulting in a layer seeded with a population of microbial cells approximately estimated at 2.4x10^5 CFU/mL, which was distributed in a volume of 25 ml in plates Petri dish measuring 20x100 mm (BrCAST 2021).

An aliquot of 200 µl of the prepared fluconazole and EFA solutions were distributed in stainless steel cylinders, respecting the 3x3 design and triplicate for each microorganism studied (Anvisa 2024a). After 24 h of incubation at 35 ± 2 °C, zones of inhibition were obtained, which were measured in millimeters with the aid of a caliper, and the mean, standard deviation, linearity and MLC determination were calculated in the software Excel® (2016 Version, Microsoft Corporation) (BrCAST 2021; Chandrasekar et al. 2015).

Plates were also made in triplicate to evaluate the effect of 1% ethanol, 1% SLS and the combination of 1% ethanol/1% SLS on the antifungal activity of EFA, in addition to ASB sterility and fertility controls for each microorganism tested.

Development and characterization of liquid soap quality

For the pharmacotechnical development of aroeira-do-sertão liquid soap (SLS), edetate disodium was dissolved and sodium metabisulfite in glycerol. Soon after, phenoxyethanol, water, sodium lauryl ether sulfate and cocamide diethanolamine were added. The pH was adjusted with lactic acid to 4.0 and the viscosity was adjusted by adding sodium chloride solution. Finally, EFA was incorporated into the preparation and the volume was made up with water in a graduated cup. The details of the qualitative and quantitative composition of the SLS are explained in Table 1.

SLS quality control was carried out by evaluating appearance, color, odor and pH, using the techniques already presented (Anvisa 2024a).

Antifungal activity of liquid soap

The antifungal activity of the soaps was evaluated using the same technique presented for the EFA, with the exception of the sample preparation conditions, where pure soap (100%) and serially diluted soap (50 and 25%) in water were used. Controls of 1% ethanol and base soap were used for the SLS assay.

All results obtained for the product under development were compared to those of a liquid soap for gynecological use containing aqueous extract of *S. terebinthifolia* (aroeira-da-praia - PLS) purchased at a community pharmacy in the city of Fortaleza, Brazil.

RESULTS AND DISCUSSION

Plant drug processing and characterization

The vegetable powder obtained was classified as moderately coarse, with an average particle size of 0.654 mm (Anvisa 2024a).

Regarding humidity, the fresh leaf had a water content of 53%, which is justified because it is the leaves and not the bark. From another perspective, the dried leaves and the pulverized vegetable drug presented a moisture percentage of 5.6 and 8.6%, comparable to the results of the same analysis using leaves from shoots and shoots as samples (Galvão 2013).

The total ash content in adult leaves was 8%, while other authors revealed that for shoot and shoot leaves it was 7 and 5.3%, respectively (Galvão 2013).

Phytochemical approach demonstrated the

Table 1. Composition of aroeira-do-sertão liquid soap

INCI ingredient	Function	Amount
EFA	Active pharmaceutical ingredient	2.5 g
Glycerin	Humectant	5 g
Disodium EDTA	Chelating agent	0.1 g
Phenoxyethanol	Antimicrobial preservative	0.5 g
Sodium metabisulfite	Antioxidant preservative	0.5 g
Sodium lauryl ether sulfate	Anionic surfactant	31 g
Cocamide diethanolamine	Surfactant, foam stabilizer and thickener	4 g
Lactic acid	pH adjuster (acidulant)	qs pH 4.0
Sodium chloride 25%	Viscosity inducer (thickening agent)	qs compatible viscosity
Aqua	Solvent	qsp 100 ml

Caption: EFA: fluid extract of aroeira-do-sertão; INCI: International Nomenclature of Cosmetics Ingredients. Source: Author.

presence of saponins, reducing sugars, flavonoids and tannins in the vegetable drug. These results are similar to those presented by other authors for aroeira-do-sertão (Galvão 2018). The qualitative identification of the chemical groups present in the plant drug suggests the use of polyphenols – such as TP and TT – as active markers for monitoring the quality of the EFA.

Production and quality control of plant extract

The EFA obtained has high consistency, intense dark red color, homogeneous appearance and characteristic odor, as seen in Figure 1.

The pH analysis confirmed the acidic nature of EFA from the leaves of the adult plant, with a pH value of 3.89. This pH allows the safe use of EFA in feminine intimate hygiene products, as it is within the physiological range of gynecological pH (3.5 to 4.5), in addition to being important for maintaining the effectiveness and stability of the preparation (Kalia et al. 2020).

Other authors demonstrated a pH of 4.66 for the fluid extract of shoot leaves. The greater acidity of EFA can be explained by the higher content of polyphenols when compared to the derived products studied by the authors, which contributes to reducing the pH of this vegetable derivative (Dall and Archela 2013; Galvão 2018).

The investigation of the phytochemical profile of EFA showed the presence of reducing sugars, flavonoids and tannins, similar to what was observed by Galvão (2018). Flavonoids were detected in the methanol fraction from column chromatography, making this purification step necessary prior to the colorimetric reaction to identify the phytochemical group mentioned due to the presence of chlorophyll, whose color makes it difficult to visualize the positivity of the test.

The analytical method used to determine the content of TP and TT showed good accuracy and linearity, with a correlation coefficient at 718 nm of 0.9953, in addition to adequate sensitivity and few interferences (Anvisa 2017; Galvão 2018). The repeatability analysis showed a DPR of 1.97%, confirming the accuracy of the technique.

Analysis of the content of active markers in EFA showed a concentration of 189 (±0.0306) mg EAG/g of EFA from TP, with only 9.43% corresponding to TT.

Although the TP content in EFA is higher than that present in leaf extracts from shoots – 134.59 (± 3.64) mg EAG/g –, the TP/TT ratio is higher in leaf extracts from young individuals (85.62%) than in the leaves of the adult plant, because tannins are metabolites that tend to accumulate in the bark in the adult stage of the plant (Galvão 2018; Dong et al. 2022). For this reason, more studies are needed to elucidate the best conditions for producing aroeira-do-sertão extracts from the leaves (Tanga 2018).

Antifungal activity of the plant extract

The antifungal activity evaluation assay confirmed the effectiveness of EFA against the strains tested by the formation of inhibition zones, which is the region with the absence of colonies or microbial film, as seen in Table 2.

As the diameter of inhibition halo formed is proportional to increase in the concentration of standard or extract, a linear regression was performed, using the logarithm of the concentrations of the standard or EFA (independent variable, in percentage) and the diameters of the halos (dependent variable, in mm).

The MLC, defined as the lowest concentration capable of producing an inhibition zone measurable, was determined as 0.5% (w/v) for *C. albicans*, *C. parapsilosis* and *C. tropicalis* and 0.25% (w/v) for *C. krusei*, which is more sensitive for genetic and metabolic reasons (Gong et al. 2018).

Figure 1. Fluid extract and liquid soaps.
A: fluid extract of adult aroeira-do-sertão leaves (EFA); **B**: liquid soap with 2.5% (w/v) fluid extract of *Astronium urundeuva* (SLS); **C**: commercial liquid soap containing aqueous extract of *Schinus terebinthifolia* (PLS). Source: Author.

Table 2. Mean and standard deviation of inhibition halos obtained from the antifungal action of the plant extract and liquid soaps.

Sample	*C. albicans* (mm±sd)	*C. krusei* (mm±sd)	*C. parapsilosis* (mm±sd)	*C. tropicalis* (mm±sd)
AF 0.5%	16±0.1	19±0.1	16±0.1	16±0.1
AF 0.25%	13±0.1	16±0.1	13±0.1	13±0.1
AF 0.125%	10±0.1	13±0.1	10±0.1	10±0.1
EFA 2%	11±0.1	13±0.1	11±0.1	11±0.1
EFA 1%	9±0.1	11±0.1	9±0.1	9±0.1
EFA 0.5%	7±0.2	9±0.1	7±0.2	7±0.2
OH Control 1%	0	0	0	0
Lauryl 1%	7±0.2	12±0.1	7±0.2	7±0.2
OH/Lauryl 1%	7±0.2	12±0.1	7±0.2	7±0.2
SLS 100%	11±0.1	13±0.1	11±0.1	11±0.1
SLS 50%	9±0.1	11±0.1	9±0.1	9±0.1
SLS 25%	7±0.2	9±0.1	7±0.2	7±0.2
Base soap control	10±0.1	10±0.1	10±0.1	10±0.1
PLS 100%	8±0.1	10±0.1	8±0.1	8±0.1
PLS 50%	7±0.2	8±0.1	7±0.2	7±0.2
PLS 25%	0	7±0.2	0	0

AF: fluconazole; EFA: fluid extract of aroeira-do-sertão; 1% OH control: 1% ethanol negative control; Lauryl 1%: negative control sodium lauryl sulfate 1%; Control OH 1%/Lauryl 1%: Negative control with the combination of 1% ethanol and 1% sodium lauryl sulfate; SLS: aroeira-do-sertão soap; PLS: aroeira-da-praia soap. Source: Author.

As *C. albicans*, *C. parapsilosis* and *C. tropicalis* exhibited similar antifungal sensitivity in the standard and sample used, this suggests the use of EFA not only in infections caused by *C. albicans*, but also in infections caused by other species of the genus *Candida* (Willems 2020).

Considering that a concentration of 0.5% of EFA is effective against all pathogens studied, a five times higher concentration of EFA was used in the finished product (SLS), resulting in a final concentration of 2.5% (w/v).

The choice of a 2.5% (w/v) EFA concentration in the formulation was not based on a specific value reported in the literature, but rather on the experimental MLC obtained in the present study (0.5% w/v for most tested *Candida* species). A fivefold increase over the MLC was adopted to ensure that the final product maintains antifungal efficacy even in the presence of possible dilution or interference during use, such as contact with vaginal secretions, organic matter, and the rinsing process. This safety margin approach is consistent with formulation practices for topical antimicrobial products, in which concentrations above the in vitro inhibitory threshold are employed to guarantee in vivo performance (Pereira et al. 2022).

Development and characterization of liquid soap quality

SLS presented as a homogeneous and transparent solution, with a yellow to light brown color, a characteristic odor, and high consistency. In contrast, PLS had a homogeneous, milky appearance (due to the presence of ethylene glycol distearate), with a pink color, a notably perfumed odor (from added fragrances), and a consistency superior to that of SLS (due to the addition of carbomer). Figure 1 presents the macroscopy of the products studied. The pH analysis revealed values of 3.96 and 4.89 for SLS and PLS, in that order. The continuous use of products outside the pH of the female intimate region can alter the concentration of hydronium ions in this anatomical site and favor the emergence of opportunistic infections for *Gardnerella vaginalis* or *Trichomonas vaginalis* (Kalia et al. 2020). It is worth noting that this possibility is only being associated with the PLS pH value exceeding the maximum (pH 4,5) described as physiological, with no direct causal relationship being established between the increase in pH and the emergence of the aforementioned infections.

Antifungal activity of liquid soap

The test to evaluate the antifungal activity of liquid soaps revealed that both SLS and PLS showed antifungal action against the *Candida* strains tested, with a sensitivity profile similar to that seen for EFA, as shown in Table 2.

SLS showed an antifungal effect very similar to EFA, which should not have happened, since SLS

is five times more concentrated than the defined extract MLC. What may have happened is that the greater viscosity of the product may have hindered its diffusion through the agar, underestimating its antifungal effect (Pereira et al. 2022).

Additionally, the difficulty in accurately pipetting a product containing a surfactant may have further contributed to underestimating the antifungal activity of the tested product, highlighting the need to evaluate equal concentrations of EFA and SLS to allow a more reliable comparison (Coutinho et al. 2021).

The antifungal activity observed in the negative controls and in the base soap can be explained by the presence of certain excipients with intrinsic antimicrobial properties, particularly surfactants such as sodium lauryl ether sulfate and cocamide diethanolamine, which have been reported to cause disruption of fungal cell membranes by reducing surface tension and solubilizing lipid components (Bannach et al. 2022; Pereira et al. 2022).

This effect, although relevant, is generally of lower intensity and shorter duration compared to the activity of plant-derived polyphenols and tannins present in the *A. urundeuva* extract, which act through multiple mechanisms, including inhibition of cell wall synthesis, protein precipitation, and oxidative stress induction (Oliveira et al. 2017; Hsu et al. 2021).

Thus, the incorporation of the extract into the base soap not only enhances the antifungal spectrum but also increases potency, as demonstrated by the larger inhibition halos obtained with SLS compared to the base alone. Additionally, the use of the extract allows the product to act synergistically, combining the detergent effect of the surfactants with the targeted antifungal action of the phytochemicals, which may contribute to a more effective prophylaxis and management of vulvovaginal candidiasis.

PLS exhibited lower antifungal activity against the yeasts tested, with smaller diameters of inhibition halos. This may be related to the composition of excipients in the base soap, whose quantitative information is unknown, as well as the concentration of the aqueous extract of aroeira-da-praia in the preparation and the content of its phytotherapeutic complex, which is responsible for the biological effect.

Therefore, the carrying out of the present work allowed the development of a liquid soap for gynecological use with proven *in vitro* antifungal action against pathogens of the genus *Candida* from an extract characterized from the leaves of adult specimens of aroeira-do-sertão.

The present study successfully developed a gynecological liquid soap formulated with 2.5% (w/v) *A. urundeuva* leaf fluid extract, characterized by a high content of polyphenols and relevant in vitro antifungal activity against *C. albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*.

These findings support the potential application of the developed product as a prophylactic and adjuvant tool in the management of vulvovaginal candidiasis. Further studies, including *in vivo* evaluations and stability assessments, are recommended to confirm its clinical applicability and ensure long-term efficacy.

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AUTHOR'S CONTRIBUTIONS

Conceptualization: SF and MAB; Methodology: SF and MAB; Formal analysis: JFR; Investigation: JFR; Resources: SF and MAB; Writing - Original draft preparation: JFR; Writing - Review and editing: SF; Visualization: JFR; Supervision: SF; Project administration: JFR; Funding acquisition: SF and MAB. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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