The Enhanced Activity of a Plant Mixture from the Brazilian Caatinga Biome against Venereal Trichomonads Confirms the Traditional Use

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Abstract: Women living in the semi-arid region of Caatinga in the northeast of Brazil report the use of plant mixtures to treat diseases in the genitourinary tract. Plant extracts were obtained from barks to simulate traditional use. The anti-trichomonads activity as well as the cytotoxic effect of plant extracts were tested. Herein, we confirmed this traditional knowledge by testing plants aqueous extracts against Trichomonas vaginalis and Tritrichomonas foetus, the etiologic agents of human and bovine trichomoniasis. All plant extracts were active individually against at least one trichomonads species except for Prosopis juliflora and Amburana cearensis. Cedrela sp. was the most active against both trichomonads species. Finally, a mixture of plants used in traditional medicine was evaluated for activity. A mixture containing extracts of the plants Ximenia americana, Anadenanthera colubrina var. cebil, Myrciadodron urundeuva, Sideroxylon obtusifolium, and Amburana cearensis was active against the two trichomonads. This finding confirms the traditional practice by women living in the Caatinga region of using a mixture of plants during sitz baths to treat vaginal infections. Altogether, these results highlight the ethnopharmacological use of Cedrela sp. and of the plant mixture for the treatment of venereal diseases by Caatinga residents.

Keywords: anti-Tritrichomonas foetus; anti-Trichomonas vaginalis; bovine trichomoniasis; Caatinga biome; human trichomoniasis; plant extracts; traditional medicine; Southern America

1. Introduction

Trichomonas vaginalis causes human trichomoniasis, the most common non-viral sexually transmitted infection (STI) in the world [1], associated with complications and the transmission facilitation of HIV. Metronidazole and tinidazole are drugs approved by the FDA (USA) for the treatment; however, they may cause adverse effects and increasing drug resistance has leaded to therapeutic failures [2]. Bovine trichomoniasis is caused by Tritrichomonas foetus transmitted by coitus. The parasite survives in raw and processed bull semen, which allows its transmission via artificial insemination, causing significant economic losses. There is no FDA-approved drug to treat bovine trichomoniasis and the vaccines available are still under optimization [3].

The Caatinga, a semi-arid biome in the northeast of Brazil, retains diversity of plants used as popular medicine. Elevated temperatures and seasonally dry forests due to the irregular rainfall regime and shrubby, spiny vegetation characterize this biome [4]. This
region is known as an area of low economic development; as a consequence, the population has low access to medicines and, therefore, use medicinal plants in the treatment of illnesses. Regional women report a large use of these native medicinal plants for diseases of the genito-urinary system and, importantly, use a mixture of plants during sitz baths to treat vaginal infections. In addition to these valuable reports (unprecedented in the formal literature), studies have shown the traditional use and have confirmed the in vitro activity of plants including Anadenanthera colubrina (Vell.) Brenan, Commiphora leptophloeos, and Myracrodruon urundeuva to treat vaginal candidiasis, gonorrhea, and HIV infection [5–7]. The use of the plant Ximenia americana by healers to treat STIs was also described [8,9]. Considering this traditional knowledge, this study aimed to determine the anti-Trichomonas vaginalis and anti-Trichomonas foetus activities of extracts from Ximenia americana, Anadenanthera colubrina var. Cebil, Myracrodruon urundeuva, Schinopsis brasiliensis, Cedrela sp., Commiphora leptophloeos, Hymenaea courbaril, Sideroxylon obtusifolium, and Amburana cearensis. Moreover, we showed that a mixture of plant extracts following the traditional medicine methods enhanced the anti-trichomonads activity.

2. Materials and Methods

2.1. Plant Extracts

The plants X. americana, A. colubrina var. Cebil, M. urundeuva, S. brasiliensis, Cedrela sp., C. leptophloeos, H. courbaril, S. obtusifolium, and A. cearensis were collected at Parque Nacional do Catimbau (PARNA do Catimbau), Pernambuco, Brazil (8°37′S 37°8′W) in February 2017 under SISGEN authorization A08E18B. The authors confirm that the authority designated Chico Mendes Institute for Biodiversity Conservation (ICMbio) granted permission through the System of Authorization and Information on Biodiversity (SISBIO) with the authentication code n° 26743-1. Exsiccates were prepared and the specimen was incorporated into the Dárdano de Andrade Lima herbarium from the Instituto Agronômico de Pernambuco, Recife, Brazil (IPA-PE): A. cearenses voucher number 95185, H. courbaril voucher number 84888, X. americana voucher number 96261, S. obtusifolium voucher number 84076, C. leptophloeos voucher number 84037, S. brasiliensis voucher number 95154, A. colubrina var. Cebil voucher number 80351, Cedrela sp. voucher number 84110, and M. urundeuva voucher number 90471. The crude extracts were obtained from barks using aqueous maceration for 24 h at room temperature based on the traditional methods.

2.2. Screening of Anti-Trichomonads Activity and Determination of IC₅₀ Values

The T. vaginalis ATCC 30236 isolate and T. foetus TFK isolate obtained by Dr. H. Guida (Embrapa, Rio de Janeiro, Brazil) from the urogenital tract of a bull were used in this study. Parasites were cultured in TYM medium (trypticase-yeast extract-maltose) with a pH of 6.0 and 7.2, respectively, and were supplemented with 10% inactivated bovine serum (purchased from Cripion Biotechnology, São Paulo, Brazil). The screening was performed in 96-well microplates. The plant extract concentrations used were 1.0 mg/mL and the trophozoites were added at a final density of 2.0 × 10⁵/mL, maintained at 37 °C for 24 h in 5% CO₂. Two controls were conducted: parasites only and metronidazole (100 µM). The activity was determined by assessing the motility and morphology of parasites compared with the negative control by counting with a hemocytometer using trypan blue dye exclusion (0.2%, v/v). Viability was determined as the percentage of viable trophozoites compared to the negative control (100% viability). The active extracts in the screening assay had the half-maximal inhibitory concentration (IC₅₀) value determined with concentrations ranging from 1.0 to 0.0078 mg/mL via serial dilution. In addition, the activity of a mixture of extracts from the plants X. americana, A. colubrina var. cebil, M. urundeuva, S. obtusifolium, and A. cearensis (1:1:1:1:1) at 1.0 mg/mL was tested as described.

2.3. Effect of Plant Extracts on Trichomonads Kinetics Growth Assays

Parasite suspensions, at a final density of 2.0 × 10⁵ trophozoites/mL, were incubated with the extracts at their IC₅₀ value. The parasites were counted with a hemocytometer
using trypan blue (0.2%) after 2, 4, 6, 12, 24, 48, 72, 96, and 120 h of incubation. Results were expressed as the number of viable trophozoites per milliliter.

2.4. Cytotoxicity Assay by MTT Assay

Human vaginal epithelial cells (HMVII) purchased from the European Collection of Authenticated Cell Cultures (ECACC, Porton Down, Wiltshire, England) were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum and 25 µg/mL penicillin at 37 °C and 5% CO₂. Briefly, 1.5 × 10⁴ cells per well at the fifteenth passage were seeded in 96-well microplates for 48 h; the medium was replaced with fresh medium containing (or not, in the case of the control condition) active extracts in the IC₅₀ range (1.0–0.3 mg/mL). Triton X-100 0.2% was used as a positive control. The plates were incubated for 48 h. After this time, a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltriazolium bromide (MTT) (0.5 mg/mL) was added and incubated for 1 h at 37 °C. MTT was removed and the insoluble purple formazan was dissolved in dimethyl sulfoxide (DMSO). The amount of reduced MTT was measured as 570 nm [10].

2.5. Thin Layer Chromatography (TLC)

The extracts were applied to TLC plates (Silica gel 60 F₂₅₄, Merck, Darmstadt, Germany) and developed using butanol, acetic acid, and water (5:1:4) as mobile phase. The plates were visualized under UV light (254 and 365 nm, Handheld UV Lamp Model 9403E, BioAmerica Inc., Miami, FL, USA) and revealed with different chemical sprays: natural reagent followed by polyethylene glycol was used to detect flavonoids; ninhydrin for amines and amino acids; anisaldehyde sulfuric for steroids, terpenoids, and saponins; and iodine vapor for alkaloids [11].

2.6. Statistical Analysis

All experiments were performed, at least, at three independent times (three different cultures, n = 3), in triplicate. The IC₅₀ and half maximal cytotoxic concentration (CC₅₀) values were determined using GraphPad Prism6 software version 8.0.2 (263) through a non-linear regression model. Results were expressed as means ± SD. Statistical analysis was conducted using Student’s t-test for comparison between two groups, test and control (only parasites). Statistical significance was considered at p < 0.05.

3. Results

3.1. Plants X. americana, M. urundeuva, S. brasiliensis, C. leptophloeos, and H. courbaril Aqueous Extracts Were Active against T. vaginalis and T. foetus

Based on ethnopharmacological data from residents of Caatinga, as well as the literature information, the plants investigated in this study were chosen to reproduce the form of use in traditional medicine, by testing the plants aqueous extract (Table 1).
Table 1. Traditional use of plants from Caatinga biome and determination of the IC<sub>50</sub>, CC<sub>50</sub>, and SI values of the plant extracts with anti-trichomonads activity.

<table>
<thead>
<tr>
<th>Plant Scientific Name and Family</th>
<th>Plant Popular Name</th>
<th>Voucher</th>
<th>Popular Use (Reference)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedrela sp. P. Browne—Meliaceae</td>
<td>Cedar</td>
<td>IPA 95.539</td>
<td>Bark infusion used against cold and flu (PC). Used to treat liver diseases, diarrhea, fever, chronic infantile dysentery, intestinal helminths, and inflammation [12]</td>
<td>0.68</td>
<td>0.74</td>
<td>0.75</td>
<td>1.10&lt;sup&gt;a&lt;/sup&gt;; 1.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Commiphora leptophloeos (Mart.) J.B.Gillett—Burseraceae</td>
<td>Imburana or Imburana-de-cambão *</td>
<td>IPA 95.547</td>
<td>Bark infusion used against cold and flu and for wound washing (PC). Used to treat inflammation and infections [13]</td>
<td>N.D.</td>
<td>0.77</td>
<td>0.61</td>
<td>0.80</td>
</tr>
<tr>
<td>Hymenaea courbaril L.—Fabaceae</td>
<td>Jatobá *</td>
<td>IPA 95.536</td>
<td>Bark infusion used against cold and flu (PC). Used to treat diarrhea, cystitis, prostatitis, malaria, and leishmaniasis [14]</td>
<td>N.D.</td>
<td>0.71</td>
<td>1.01</td>
<td>1.25</td>
</tr>
<tr>
<td>Miconodron urundeuva Allemão—Anacardiaceae</td>
<td>Aroeira *</td>
<td>IPA 95.511</td>
<td>Bark infusion used against cold and flu and for wound washing (PC). Used to treat vaginal infections [5]</td>
<td>N.D.</td>
<td>0.84</td>
<td>0.72</td>
<td>0.86</td>
</tr>
<tr>
<td>Schinopsis brasiliensis Engl.—Anacardiaceae</td>
<td>Brauna or Barauna *</td>
<td>IPA 95.542</td>
<td>Bark infusion used for digestive problems (PC). Used to treat inflammation, diarrhea, and as an antiseptic [15]</td>
<td>N.D.</td>
<td>0.87</td>
<td>0.79</td>
<td>0.91</td>
</tr>
<tr>
<td>Ximenia americana var. microphylla Welw.—Olacaceae</td>
<td>Ameixa recanto *</td>
<td>IPA 95.524</td>
<td>Bark infusion used for inflammation and wound washing (PC) [16]</td>
<td>N.D.</td>
<td>0.83</td>
<td>0.50</td>
<td>0.61</td>
</tr>
<tr>
<td>Sideroxylon obtusifolium (Roem. and Schult.)—Sapotaceae</td>
<td>Quixabeira *</td>
<td>IPA 95.523</td>
<td>Bark infusion used for inflammation and wound washing (PC). Used for wounds, pain, chronic inflammation, genital problems, ovarian, colon, and kidney problems, heart disease, diabetes, fever, and as an expectorant (PC)</td>
<td>Used in the mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amburana caeensis (Allemão) A.C.Sm.—Fabaceae</td>
<td>Umburana *</td>
<td>IPA 95.537</td>
<td>Bark infusion used against cold and flu (PC). Used to treat inflammatory diseases [17]</td>
<td>Used in the mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anadenanthera colubrina var. cebil—Fabaceae</td>
<td>Angico</td>
<td>IPA 95.503</td>
<td>Bark infusion used against cold and flu (PC). Used for inflammation, diarrhea, cough, bronchitis, influenza, and toothache [18]</td>
<td>Used in the mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PC—personal communication. N.D.—not determined; <sup>a</sup>—selectivity index (SI) value for the crude extract of barks of Cedrela sp. plant for *T. vaginalis*; <sup>b</sup>—SI value for the crude extract of barks of Cedrela sp. plant for *T. foetus*. * No English name found.
Among 10 plants collected, seven of them presented activity against the parasites, corroborating their popular use to treat ovarian or vaginal infection. Figure 1 shows the screening of ten aqueous extracts against *T. vaginalis* and *T. foetus*. Considering the plant *Cedrela* sp., the extract presented high anti-parasitic activity with 99.6% and 100% of the reduction in *T. vaginalis* and *T. foetus* viability, respectively. The plants *X. americana*, *M. urundeuva*, *S. brasiliensis*, *C. leptophloeos*, and *H. courbaril* showed activity against *T. foetus*, strongly reducing the trophozoite viability by 96% (Figure 1).

![Figure 1](image-url). Effect of extracts from the barks of plants in trichomonads viability. MTZ: metronidazole as positive control (100 µM or 0.0171 mg/mL). Data are presented as mean ± standard deviation compared to control (considering the trophozoite viability as 100%). Results are representative of three independent experiments performed in triplicate assays. * means statistical significance in comparison with controls (*p* < 0.05).

3.2. *Cedrela* sp. Extract Was the Most Active against *T. vaginalis* and *T. foetus*

Based on the results of the screening, six plants that were active were chosen for the determination of the IC$_{50}$: *Cedrela* sp., *X. americana*, *M. urundeuva*, *S. brasiliensis*, *C. leptophloeos*, and *H. courbaril*. Corroborating the result of the screening, *Cedrela* sp. presented the highest anti-trichomonads activity (Table 1). Moreover, a decrease in parasite growth after 4 h of incubation with *Cedrela* sp. extract could be observed (Figure 2). All other plant extracts also reduced the *T. foetus* proliferation by 50% in 24 h. As expected, after 24 h of incubation, the untreated trophozoites (control) exhibited the classic growth peak (Figure 2).
3.2. Cedrela sp. Extract Was the Most Active against T. vaginalis and T. foetus

Based on the results of the screening, six plants that were active were chosen for the determination of the IC50: Cedrela sp., X. americana, M. urundeuva, S. brasiliensis, C. leptophloeos, and H. courbaril. Corroborating the result of the screening, Cedrela sp. presented the highest anti-trichomonads activity (Table 1). Moreover, a decrease in parasite growth after 4 h of incubation with Cedrela sp. extract could be observed (Figure 1A). All other plant extracts also reduced the T. foetus proliferation by 50% in 24 h. As expected, after 24 h of incubation, the untreated trophozoites (control) exhibited the classic growth peak (Figure 1B).

Figure 2. Effect of extracts from the barks of plants in the kinetic growth curve of (A) T. vaginalis and (B) T. foetus. Trophozoites treated with extracts of plants, at IC50 values, were counted in comparison to untreated parasites (control). The initial inoculum was 2.0 × 10⁵ trophozoites/mL. Results are presented as mean ± standard deviation of three independent experiments in triplicate. * Statistically different from control (p < 0.05).
3.3. Active Plant Extracts Showed Low Selectivity

Cytotoxicity results of the most active extracts against the vaginal epithelial cell line (HMVII), Cedrela sp., Commiphora leptophloeos, Hymenaea courbaril, Myracrodruon urundeuva, Schinopsis brasiliensis, and Ximenia americana, are shown in Table 1 (Supplementary Materials, Figure S1). The selectivity index (SI) values obtained were below 1.5, indicating that the plant extracts were nonselective to the parasites.

3.4. The Mixture of Plants Was Effective against Both Trichomonads Species

The mixture of extracts from the barks of plants X. americana, A. colubrina, M. urundeuva, S. obtusifolium, and A. cearensis (1:1:1:1) reduced the viability of the trophozoites by 79.2% and 66.9% for T. vaginalis and T. foetus, respectively (Figure 3).

Figure 3. Effect of mixture of extracts from the barks of plants X. americana, A. colubrina, M. urundeuva, S. obtusifolium, and A. cearensis (1:1:1:1) in trichomonads viability. MTZ: metronidazole as control (100 µM). Data are presented as mean ± standard deviation compared to control (considering the trophozoite viability as 100%). Results are representative of three independent experiments performed in triplicate assays. * means statistical significance in comparison with controls (p < 0.05).

3.5. Qualitative Phytochemical Screening

The preliminary qualitative phytochemical screening of the plant extracts indicated the presence of flavonoids and tannins in the extracts of P. juliflora, X. americana, A. colubrina var cebil, M. urundeuva, S. brasiliensis, and H. courbaril (Figure 4A), while alkaloids were only detected in P. juliflora (Figure 4B). As it can be observed, no amines, amino acids, terpenoids, or saponins were detected (Figure 4C,D). However, more studies are needed to evaluate and characterize the constituents of these extracts, since the technique used, TLC, is a preliminary approach.
The in vitro cytotoxicity should not be used as a general guideline and not as an exclusion factor for study of a particular compound. The SI values should be used as a general guideline and not as an exclusion factor for study of a particular compound. This selectivity was not found in the present study, but it is important to point out that the SI was calculated for a crude extract with complex composition and the SI values should be used as a general guideline and not as an exclusion factor for study of a particular compound. The in vitro cytotoxicity should not be used as a general guideline and not as an exclusion factor for study of a particular compound.
be the unique criterion to decide whether a compound should be rejected or forwarded to an animal model to continue the search for a new bioactive molecule. Moreover, previous studies showed anticancer activity and low toxicity of the plants used in this study in mouse and *Drosophila melanogaster* in vivo models [12,17,18,20,21].

Regarding the chemical composition of the extracts, the results found here are in agreement with other studies that identified the presence of polyphenols, terpenes, limonoids, and tannins in *X. americana, M. urundeuva, S. brasiliensis, Cedrela sp., C. leptophloeos*, and *H. courbaril* [22–28], and these classes of compounds have already demonstrated anti-trichomonads activities [29].

Women living in the Caatinga region use a mixture of plants during sitz baths to treat vaginal infections. Women’s reports showed that plant species with anti-trichomonads activity are used in the treatment of candidiasis, discharge, urinary tract infection, pelvic inflammation, pelvic hemorrhage, hormone replacement, menopause, menstrual cramps, and uterine wounds. These dialogues also showed that popular knowledge and the practice of traditional medicine are still very present in an isolated area with difficult access to basic health care. An ethnobotanical study in these communities is interesting, being an important tool in the rescue and enhancement of traditional knowledge, the cultural diversification of these societies, and the preservation of natural resources, especially in areas with remnants of Caatinga (data from the Bioprospecting and Conservation Nucleus of Caatinga-NBioCaat). Taking into account the popular usage, the findings in this study are relevant, since the plant extracts mixture presented high anti-*T. vaginalis* activity. The limitation of this study is the lack of chemical composition of plant extracts, which does not compromise the contribution since the aim was to reinforce the popular use of these plants, and especially the plant mixture.

5. Conclusions

Overall, the anti-*T. vaginalis* and anti-*T. foetus* activities demonstrated in this study reaffirm the importance of the traditional knowledge for the treatment of venereal diseases. The anti-trichomonads activity of the mixture containing extracts of the plants *Ximenia americana, Anadenanthera colubrina var. cebil, Myracrodruon urundeuva, Sideroxylon obtusi folium*, and *Amburana cearensis* is highlighted, thus confirming the traditional use by women living in the Caatinga region of a mixture of plants during sitz baths to treat vaginal infections. The preliminary qualitative phytochemical screening of the plant extracts indicated mainly the presence of flavonoids and tannins. In addition, this study reinforces the impact of natural products as a source of new active molecules, demonstrating the significant pharmacological potential of the plant species from the Caatinga biome.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/venereology3010002/s1. Figure S1. Determination of IC$_{50}$ values for anti-*Tritrichomonas foetus* activity (graphs B, D, F, H, J, L), except for *Cedrela* spp., which had IC$_{50}$ values determined for anti-*T. vaginalis* and anti-*T. foetus* activities (graphs A and B, respectively). Cytotoxicity of plant extracts tested against human vaginal epithelial cells (HMVII lineage) is represented in graphs C, E, G, I, K, M. Graphs showing CC$_{50}$ estimate using GraphPadPrism6 software version 8.0.2 (263) through a non-linear regression model. Bars represent cell viability as mean ± standard deviation obtained by MTT assay as described in Material and Methods.

**Author Contributions:** Conceptualization, N.L.F.S. and T.T.; methodology, N.L.F.S., P.d.B.V. and M.V.d.S.; resources, M.V.d.S.; data curation, M.V.d.S.; writing—original draft preparation, N.L.F.S. and P.d.B.V.; writing—review and editing, A.J.M. and T.T.; supervision, T.T.; project administration, A.J.M. and T.T.; funding acquisition, A.J.M. and T.T. All authors have read and agreed to the published version of the manuscript.

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