Novel Treatment Approaches to Combat Trichomoniasis

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Trichomoniasis is a neglected sexually transmitted infection (STI) caused by Trichomonas vaginalis, a flagellate protozoan responsible for a prevalence of 110.4 million cases and 156.0 million rate of incidence. The last estimative from the World Health Organization (WHO) demonstrated the incidence rate for trichomoniasis across the globe, highlighting the African Region with the highest rates, followed by America, Western Pacific, Eastern Mediterranean, South-East Asia, and last, the European region. Although most cases are asymptomatic, complaints such as pruritus, vaginal discharge, irritation, and odor are still reported. The long-lasting infection of T. vaginalis, which can persist for months to years, may lead to severe complications such as the premature delivery and low weight of newborns, infertility, pelvic inflammatory disease, and a positive association with the onset of cervical and prostate cancer.

trichomoniasis

1. Introduction

Trichomoniasis is a neglected sexually transmitted infection (STI) caused by *Trichomonas vaginalis*, a flagellate protozoan responsible for a prevalence of 110.4 million cases and 156.0 million rate of incidence [1][2]. The last estimative from the World Health Organization (WHO) demonstrated the incidence rate for trichomoniasis across the globe, highlighting the African Region with the highest rates, followed by America, Western Pacific, Eastern Mediterranean, South-East Asia, and last, the European region [2]. Although most cases are asymptomatic, complaints such as pruritus, vaginal discharge, irritation, and odor are still reported. The long-lasting infection of *T. vaginalis,* which can persist for months to years, may lead to severe complications such as the premature delivery and low weight of newborns, infertility, pelvic inflammatory disease, and a positive association with the onset of cervical and prostate cancer [3][4]. Moreover, a bidirectional relationship with human immunodeficiency virus (HIV) transmission and acquisition has already been described, where patients infected with *T. vaginalis* are 1.5 times more likely to acquire HIV than those not infected [5].

According to the last STI treatment guidelines from the Centers for Disease Control and Prevention (CDC-USA), the only approved drugs—metronidazole (MTZ) and tinidazole—belong to the 5-nitroimidazole class. The main treatment is based upon MTZ, with the recommended regimen of 500 mg orally two times/day for 7 days among women, and 2 g orally in a single dose among men. The alternative treatment relies on tinidazole 2 g orally in a single dose. Moreover, CDC recommends testing for other STIs and abstaining from sex until all the involved are properly treated [6]. The complementary intravaginal treatment with boric acid, paromomycin sulfate, povidone

iodine, and furazolidone appears to show some efficacy, but lesser than approved drugs [7]. However, high rates of treatment resistance and the mechanisms that activate this process have already been described in the literature, and these emphasize the importance of developing alternative therapies to prevent the spread of this infection, as well as the associated comorbidities [8].

2. Synthetic Compounds

The synthesis of new natural product-based compounds is another focus of researchers in the development of alternatives against trichomoniasis. Betulinic acid derivatives eliminated 100% of trophozoite's viability after adding an amide group with a piperazine (compound **4**) and one piperazine group bonded to a BOC group (compound **3**). Compound **4** presented lower MIC values, ranging between 25 and 50 µM, against *T. vaginalis* fresh clinical isolates [9]. Moreover, another study investigated the tricomonacidal actions of different ursolic and betulinic acid derivatives against fresh clinical and ATCC isolates. At 25 µM, the compound 3-oxime-urs-12-en-28-oic-ursolic acid showed 100% trichomonacidal activity against most of the tested isolates, including the MTZ-resistant isolate [10]. Phenanthrene-based compounds, in their free form and associated with metals, were synthesized and demonstrated potent anti-*T. vaginalis* activity against fresh clinical and ATCC isolates. The geometric means obtained for MIC/IC_{50} of 1,10-phenanthroline-5,6-dione (phendione) were 42.04/6.57 µM, while silver–phendione presented 21.02/2.84 µM, and copper–phendione demonstrated 8.84/0.87 µM, lower than those obtained for MTZ (9.71/1.64 µM). In addition, a synergic interaction between copper–phendione and MTZ was reported [11]. Three synthetic analogues of curcumin, 1,5-diphenylpenta-1,4-dien-3-one (**3a**), 1,5-bis(2-chlorophenyl)penta-1,4-dien-3-one (**3e**), and 2,6-bis(2-chlorobenzylidene)cyclohexanone (**5e**), demonstrated antiparasitic effects, with MIC/IC_{50} values of 80/50 µM, 90/50 µM, and 200/70 µM, respectively [12]. In efforts to demonstrate the applicability of a colorimetric technique for detecting trichomonacidal activity, the authors identified a promising candidate from a vast library of 812 compounds. An inhibitor of methionine aminopeptidase 2, described as fumagillin, was one of the hit components identified via in vitro assay, with an IC_{50} of 0.26 µM and with target action confirmed by in silico assays [13].

Drug repositioning is also described as an alternative in the search for trichomonacidal agents. The membrane-active synthetic lipid analogue miltefosine is a known antimicrobial that was investigated due to its anti-*T. vaginalis* activity. The compound exhibited an IC_{50} of 14.5 µM and showed alterations in trophozoite morphology, such as rounded and wrinkled cells, membrane blebbing, and intense vacuolization and nuclear condensation [14]. The topical use of boric acid is already described in the STI guidelines as an alternative to treat diseases in the female genital tract [6]. Investigations into the trichomonacidal activity of boric acid continue to incite the interest of researchers, and the MLC (minimum lethal concentration) occurred in a range between 0.3–0.6%, as tested in long-term-grown and fresh clinical *T. vaginalis* isolates [15]. The proton pump inhibitors omeprazole, lansoprazole, pantoprazole and rabeprazole used in therapeutics also showed remarkable anti-*T. vaginalis* activity, with IC_{50} values in the sub-micromolar range of 0.1216 µM, 0.1218 µM, 0.0756 µM and 0.1057 µM respectively, being 1.9–3.1 times more active than MTZ [16].

Another classical case of drug repositioning occurs with tetracycline (TET), a broad-spectrum antibacterial with activities against intra- and extracellular protozoa. In that paper, the in vitro assessment of the anti-trichomonads effect showed a cytotoxic effect with TET at 700 µg/mL (4 h), which induced structural changes similar to apoptosis as well as the activation of specific transcriptome pathways [17]. Octenisept® (Schülke and Mayr GmbH, Vienna, Austria), a combination of octenidine dihydrochloride with phe-noxyethanol, is known for broad-spectrum antimicrobial activity. The authors demonstrated promising anti-*T. vaginalis* activity with EC_{50} values ranging from 0.68 to 2.11 g/mL after 30 min of incubation [18]. Nitazoxanide, known for its anti-parasitic activity, showed activity against MTZ-resistant and MTZ-sensitive *T. vaginalis* isolates. After 24 h of incubation, the MLC values of nitazoxanide for both isolates tested were 50 and 6 µg/mL, while MTZ exhibited MCLs of 100 and 12 µg/mL, respectively [19]. Secnidazole, approved for the treatment of bacterial vaginosis, was investigated for trichomonacidal activity using fresh clinical isolates, and demonstrated a median MLC of 1.6 µg/mL, while MTZ exhibited a medium value of 6.3 µg/mL [20]. Clotrimazole (CTZ) and its zinc salt complexes were explored, and the superior effect was observed for the $[Zn(CTZ)_2(Ac)_2]$ complex, with IC_{50} value of 4.9 µM. Moreover, the authors highlighted changes in the morphology of hydrogenosomes, endoplasmic reticulum, and Golgi complex [21]. Zinc sulfate also demonstrated therapeutic effects in eight cases of MTZ-resistant trichomoniasis. Zinc (1.0%) douche with or without oral combined therapy with tinidazole for 14 or 28 days led to negative vaginal wet smear [22]. More than a thousand approved drugs or compounds in clinical trials were screened against MTZ-sensitive and -resistant *T. vaginalis* under aerobic and anaerobic conditions. In this sense, disulfiram and nithiamide demonstrated trichomonacidal effects when used alone, with disulfiram presenting an IC_{50} (µM) value (aerobic/anaerobic) of 0.06/0.09 for MTZ-sensitivity and 0.10/1.52 for MTZ-resistance, while nithiamide showed 1.33/0.78 and 5.88/1.51, respectively. A better combinatorial effect with MTZ was found for albendazole and coenzyme B12, under aerobic and anaerobic conditions [23].

The class of 5-nitroimidazoles is still under investigation regarding novel routes of administration. Thermosensitive and mucoadhesive hydrogels have been developed, aiming for the topical delivery of MTZ. Through in vitro viability analysis, authors confirmed that MTZ (0.7 wt. %) combined with pluronic® F127 (20 wt. %) and chitosan (1 wt. %) preserved anti-*T. vaginalis* activity and still allowed the control of drug release over time [24]. The activity of MTZ against trichomonads was also maintained after the process of complexation with methylated β-cyclodextrin, where the MTZ/RAMEB (randomly methylated β-CD) and MTZ/CRYSMEB (low methylated β-CD) complexes showed the same activity profiles in trophozoites viability, in the range of 0.01 to 10 µg/mL [25]. Furthermore, this class arouses interest in terms of finding a derivative with increased effectiveness that is able to escape from resistance pathways related to MTZ. Through the derivatization of the nitroimidazole carboxamide scaffold, a library of re-examined “old” nitroimidazoles was evaluated against *T. vaginalis* trophozoites. The authors described EC_{50} values in the range of 0.6 to 1.4 µM for new compounds, comparable to MTZ EC_{50} (0.8 µM) [26]. Chlorinated MTZ was also listed as a promising alternative for trichomoniasis therapy, presenting IC_{50} values of 0.006 and 0.24 µM against sensitive and resistant isolates, while MTZ presented IC_{50} values of 0.068 and 0.49 µM, respectively [27]. A vast library of structurally distinct 5-nitroimidazoles was developed to evaluate the microbial potential against bacteria and protozoa. Of 378 compounds, 40% of them demonstrated remarkable anti-*T. vaginalis* activities that were superior to MTZ.

In vitro and in silico studies suggest 3-alkoxy-5-nitroindazoles as promising starting scaffolds for the further development of novel compounds. Four 3-alkoxy-5-nitroindazole derivatives inhibited parasite growth by more than 50% at 10 µg/mL. Two compounds showed remarkable activity at the lowest dose tested (1.0 µg/mL), inhibiting parasite growth by nearly 40% with non-cytotoxic profiles at the concentrations assayed, showing a fair antiparasitic selectivity index (SI > 7.5) [28]. In addition, another series of nitroindazoles showed promising anti-*T. vaginalis* activity, especially with two derivatives, 2-Benzyl-3-(2-hydroxyethoxy)-5-nitro-2*H*-indazole and 2-Benzyl-3-(3-hydroxypropoxy)-5-nitro-2*H*-indazole, the last one also being active against an MTZ-resistant isolate (IC_{50} MTZ: 5.78 µM) with an IC_{50} value of 9.11 µM, and IC_{50} 7.25 µM against the MTZ-sensitive isolate [29]. The same research group synthesized new series of 1,2-disubstituted indazolinones, 3-(aminoalkoxy)indazoles, and the 3-(alkylamino)indazoles compounds presented values of IC_{50} < 50 µM, with attention drawn to four derivatives that, although less active than MTZ (IC_{50} = 1.4 µM), showed interesting activities against the parasite, with IC_{50} values < 16 µM. The 3-(aminoalkoxy)indazoles (compound **27**) was the most active, with IC_{50} values of 5.6 and 8.5 µM against MTZ-sensitive and -resistant isolates, respectively [30]. Recently, Ibáñez-Escribano et al. [31] continued their efforts on prospecting potent anti-*T. vaginalis* compounds by synthesizing a series of 11 3-(ω-aminoalkoxy)-1-benzyl-5-nitroindazoles, starting from 1-benzyl-5-nitroindazol-3-ol. Six derivatives showed IC_{50} < 20 µM against the MTZ-sensitive isolate. Two compounds (**6** and **10**) displayed better IC_{50} values (1.3 and 0.5 µM respectively) against MTZ-resistant isolates than that of the reference drug (IC_{50} MTZ = 3.0 µM), and IC_{50} values 19.2 and 2.5 µM against MTZ-sensitive isolates, respectively. It is important to note that all nitroindazoles compounds active against *T. vaginalis* presented low cytotoxicity against Vero cells.

Quinoxalines have also been investigated regarding their anti-*T. vaginalis* activity. Two series of ten novel 7-nitroquinoxalin-2-ones and ten 6-nitroquinoxaline-2,3-diones with diverse substituents at positions 1 and 4 were synthesized and evaluated. 7-Nitro-4-(3-piperidinopropyl)quinoxalin-2-one (**9**) demonstrated the highest trichomonacidal activity (IC_{50} 18.26 µM) and was subsequently assayed in vivo in a murine model of trichomoniasis. Reductions of 46.13% and 50.70% in pathogenic injuries were observed in the experimental groups treated orally for 7 days with 50 mg/kg and 100 mg/kg doses, revealing the potential interesting structural cores of nitroquinoxalinones as trichomonacidal molecules [32].

In vivo analysis based on animal models of human trichomoniasis presents challenges to the standardization of reproducible infection models. An experimental primate model for *T. vaginalis* infection was developed in the pigtailed macaque (*Macaca nemestrina*), sustaining the protozoal infection for up to 2 weeks [33]. However, the use of macaque as an infection model is infeasible, because it makes the process expensive and requires a much larger structure for maintenance. Therefore, several researchers have been using mice as a vaginal infection model, and adapting those using hormones and specific human microbiota, or through the evaluation of trichomonacidal activity by infecting mice with a *Tritrichomonas foetus*. Auranofin demonstrated activity against *T. vaginalis*, with IC_{50} values of 0.7–2.5 µM and MLCs of 2.0–6.0 µM, through thioredoxin reductase inhibition. To assess the compound's ability to eliminate the parasite in a complex infection model, authors tested auranofin against the *T. foetus* and used this species to perform the in vivo infection. The trichomonacidal effect was confirmed following compound oral administration for 4 days, without any adverse effects [34]. This approach was also used by Natto [35] and Miyamoto [36] to evaluate the trichomonacidal activity of the most effective compounds.

Deazopurine nucleoside analogue 7-deaza,7-(3,4-dichlorophenyl)adenosine (FH3147) presented EC_{50} value of 0.029 μM against *T. vaginalis* [35]. The screening of compounds containing gold highlighted the anti-*T. vaginalis* activities of the derivatives (tri-n-ethylphosphine)gold(I) chloride (**4**) and (tri-n-methylphosphine)-gold(I) chloride (**10**) [36]. The use of methylene blue and light-emitting diodes was evaluated against MTZ-sensitive and -resistant *T. vaginalis* isolates. The in vivo photodynamic therapy occurred through the application of 68.1 J/cm^2 to the vaginal canals of female BALB/c mice after a pre-estrogenization procedure to enable *T. vaginalis* infection [37]. In addition, a dose of 25 mg/kg per day for four days of compound 2,2′-[α,ω-propadiylbis(oxy-1,3-phenylene)]bis-1*H*-benzimidazole cured a subcutaneous mouse model infection using *T. vaginalis* MTZ-susceptible and MTZ-refractory isolates, and the efficacy was also determined by in vitro susceptibility assay, presenting an MIC value of 9.0 μM [38].

New approaches for trichomoniasis treatment tested in humans can also be found in articles, as case reports or randomized controlled trials (RCT). The treatments and follow-ups of individual patients observed in case reports are described, with the following approach: (a) intravaginal paromomycin, 5.0 g of a 5.0% cream with concomitant oral tinidazole 1.0 g, three times daily for 14 days; (b) high-dose oral tinidazole (1.0 g, three times daily) and 4.0 g of 6.25% intravaginal paromomycin cream nightly for 2 weeks; (c) intravenous MTZ 500 mg, intravaginal boric acid 600 mg daily and liquid tinidazole 2.0 g daily for 14 days; (d) intravenous MTZ 500 mg plus MTZ vaginal gel for one week [39][40][41][42]. In addition, tinidazole has been investigated for patients with MTZ allergies, demonstrating success in desensitization protocols, with doses ranging from 3.3 to 1000 mg [43]. RCT papers related trials involving experimental groups that obtained the intervention compared to control groups, considering conventional treatments (oral MTZ 2.0 g single dose) [44]. The trichomonacidal activity of intravaginal Neo-Penotran Forte (Embil Pharmaceuticals, Istanbul, Turkey) was demonstrated following the combination of 750 mg of MTZ with 200 mg of miconazole, used once or twice a day [45]. The same active compounds were evaluated by the use of the same dose for five consecutive nights each month for 12 months, to prevent vaginal infections. However, no significant reduction in *T. vaginalis* infection was observed [46].

In another case report (NCT01018095), the authors demonstrated a superior effect using 500 mg of MTZ twice daily for 7 days in 270 patients (37% were 30 years old) [47]. The efficacy related to a single oral dose of secnidazole was demonstrated (NCT03935217), compared to the placebo group, with increased microbiological cure [48] in 147 patients randomized at an age of 36.9 years (mean). The most recent advance to date is the re-evaluation of the MTZ dose used in the treatment of trichomoniasis. In this trial, 500 mg of MTZ twice daily for 7 days (multi-dose) appeared to show better results than a 2 g single dose [49].

3. Natural Products

Historically, the therapeutic approach of natural products (NP) has been based on infusion, compression, inhalation, or sitz baths with medicinal plants. Through scientific improvement, natural supplies become rich sources of promising molecules for drug development. The technological approach is revolutionizing NP bioassay-guided isolation, together with metabolomic and genomic combined techniques, allowing the production of specific secondary metabolites [50]. In a bioactivity-guided isolation pipeline, NP of plant, animal or microbial origin are

extracted using several solvents to obtain a crude extract, proceeding to the bioguided fractionation steps until deriving a single compound responsible for the biological activity [51]. In the period from January 1981 to September 2019, an extensive review of NP as the source of new drugs showed a total number of 1602 new chemical entities and medical indications, with only two unaltered NP, seven NP derivatives, and three synthetic drugs with NP pharmacophore approved as antiparasitic drugs [52]. The process of new drug development can be costly and time-consuming, from the active discovery to the regulation of the final product by specific regulatory departments. This session highlights 33 articles, published in the last decade, that describe the use of NP as promising molecules against trichomoniasis.

Among the main challenges when using molecules produced by living organisms is the need to reproduce in vitro the most reliable natural habitat. Thus, knowledge about the region of occurrence becomes a crucial parameter. In this sense, Pistacia lentiscus L. mastic from Greece and *Ocimum basilicum* L. oil (commercially obtained) were screened against *T. vaginalis*, and their MIC values were 15 and 30 µg/mL, respectively [53]. *Phaseolus vulgaris* L. (kidney bean) lectin, obtained from Egypt, and *Nigella sativa* L. seeds/oil, acquired from a local Egyptian herb store, were evaluated against fresh clinical isolates of *T. vaginalis*. The damage to trophozoites was evaluated through ultrastructural changes, in which *N. sativa* oil and *P. vulgaris* lectin demonstrated great toxic effect at 500 µg/mL [54]. Morinda species can be recovered in tropical regions of the world, and are described by the large presence of anthraquinones. The anthraquinone lucidin-ω-isopropyl ether from *M. panamensis* Seem. roots presented anti-*T. vaginalis* activity with an IC_{50} of 1.32 µg/mL, and its potential as a metallopeptidase inhibitor has been elucidated [55]. The plants traditionally used in Northern Maputaland in South Africa were explored against several STI pathogens, such as *T. vaginalis*. Aqueous and organic extracts from nineteen plant species were screened against clinical isolates. *Bidens Pilosa* L., *Ozoroa engleri* R. Fern. and A. Fern., *Sarcophyte sanguinea* Sparrm., *Syzygium cordatum* Hochst. ex Krauss, and *Tabernaemontana elegans* Stapf presented the lowest MIC values of 1.0 mg/mL from organic extracts [56]. Eleven phloroglucinols, derived from southern Brazil *Hypericum* L. species, had activity against *T. vaginalis*, and their mechanisms of action were elucidated. In that study, a phloroglucinol derivative (isoaustrobrasilol B) presented the lowest IC_{50} value (38 µM), with the inhibition of the enzymes nucleoside triphosphate diphosphohydrolase and ecto-5′-nucleotidase activities, important to pro- and anti-inflammatory balance in the infection site [57]. Given Brazil's biodiversity, the Caatinga semi-arid region in Northeast Brazil contains several plants with activity against *T. vaginalis*. Aqueous extracts from *Polygala decumbens* A.W. Benn roots, belonging to the Polygalaceae family, eradicated trophozoite viability, and presented MIC values of 1.56 mg/mL against an MTZ-resistant isolate [58]. *Manilkara rufula* (Miq.) H. J. Lam, another plant from the Caatinga region, demonstrated trichomonacidal potential, with leaf extracts reducing 100% at 1.0 mg/mL, and bioguided fractionation of the crude extract generated several fractions and synthesized derivatives. Of all tested samples, ursolic acid showed potential activity, with MIC values of 50 and 12.5 µM against MTZ-sensitive and -resistant isolates, respectively [59]. In seeking to elucidate the anti-*T. vaginalis* activity of *M. rufula* derivatives, crude and purified saponin fractions were evaluated. The enriched saponin fraction (H100) showed MICs of 0.5 mg/mL and 1.0 mg/mL against MTZ-sensitive and -resistant isolates, respectively, with synergic interaction when 0.5 mg/mL H100 (half MIC) was associated with a sub-lethal concentration of MTZ (0.0026 mg/mL). At 0.5 mg/mL, saponin showed diverse activity rates against seven fresh clinical *T.*

vaginalis isolates, and the investigation of the mechanisms of action indicated alterations in parasite ultrastructure, with membrane damage and intracellular content disruption [60].

Indeed, the knowledge of chemical compounds produced by plants has important advantages in elucidating biomolecules activity. The presence of saponin in southern Brazilian native plants led to the trichomonacidal evaluation of butanol extract from *Ilex paraguariensis* leaves, aqueous extracts of leaves from *Quillaja brasiliensis* (A.St.-Hil. and Tul.) Mart., and saponin- and flavonoid-enriched fractions of ethanolic extracts of leaves from *Passiflora alata* Curtis, as well as the biological activity assessment of two commercial saponins. Of these samples, only flavonoid-enriched fractions from *P. alata* did not show trichomonacidal activity, and the lowest MIC value (0.025%) was demonstrated in saponins from *Quillaja saponaria* Molina and *P. alata* [61]. Another flavonoid investigated against *T. vaginalis* was quercetin from *Kalanchoe daigremontiana* Raym.-Hamet and H. Perrier. Biological assays revealed that quercetin was more effective than a crude methanolic extract of *K. daigremontiana*, with IC_{50} of 21.17 µg/mL, while the extract showed IC_{50} of 105.27 µg/mL [62]. Coumarins were investigated as trichomonacidal molecules through the evaluation of *Pterocaulon balansae* Chodat, with an anti-*T. vaginalis* MIC of 30 µg/mL and an IC_{50} of 3.2 µg/mL from the coumarin-enriched extract [63].

The anti-*T. vaginalis* activities of microbial extracts were described for several fungal families. Filtrates from southern Brazilian marine-associated fungi (*Hypocrea lixii* and *Penicillium citrinum*) revealed two samples with lower MIC, with a value of 2.5 mg/mL against clinical and long-term-grown isolates of *T. vaginalis* [64]. Complex structures produced as secondary metabolites by Basidiomycotina fungi arouse interest for the investigation of trichomonacidal action using extracts of *Amauroderma camerarium* from southern Brazil. *A. camerarium* cultivation in the medium with KNO_3 resulted in an extract with 76% anti-*T. vaginalis* activity. Proceeding with the characterization of the activity, the authors identified the protein amaurocine, with an MIC of 2.6 µM against ATCC *T. vaginalis* isolates and an MIC of 5.2 µM against fresh clinical isolates [65]. In addition, antimicrobial peptide attract attention due to their important potential use in drug development. Prophenin 2 peptide, from the porcine cathelicidin family, was cloned and expressed in *Escherichia coli*, and the authors found an anti-*T. vaginalis* activity with LD_{50} of 47.66 µM [66].

Traditional knowledge can also be explored in the study of new molecules against trichomoniasis. In this sense, a study carried out in Iran collected samples of plants used by locals for vaginal infection treatment. Indeed, *Eucalyptus camaldulensis* Dehnh., from Khostan trees, presented a trichomonacidal effect with 60 µg of extract able to abolish the parasite proliferation after 72 h of incubation [67]. In another study, several extracts from *E. camaldulensis* were tested, and the ethyl acetate fraction showed the highest rates of growth inhibition with the lowest concentration (12.5 mg/mL) in the first 24 h [68]. Considering the activity described for E camaldulensis, the leaves used for production of phenolic extract were employed in the development of a vaginal cream, together with phenolic extract from *Viola odorata* L. roots and hydroalcoholic extract from *Mentha piperita* L. leaves. The in vitro biological activity of the extract combination demonstrated that 2.5 mg *E. camaldulensis*, 0.06 mg *V. odorata*, and 1.0 mg *M. piperita* caused 100% proliferation inhibition of *T. vaginalis*; in addition, the vaginal cream was approved in all pharmacopeial tests [69]. Mbyá-Guarani indigenous knowledge was explored through in vitro evaluation of traditional plants, and two aqueous extracts from *Campomanesia xanthocarpa* O. Berg

and *Verbena* L. sp. demonstrated potent anti-*T. vaginalis* activity with an MIC of 4.0 mg/mL [70]. In Chinese cuisine and traditional medicine, *Amomum tsao-ko* Crevost and Lemarié is widely used, and several biological activities have been described. Essential oil from *A. tsao-ko* was produced and, together with one of the main components, geraniol, was evaluated against *T. vaginalis*, with MLC/IC_{50} (µg/mL) values of 44.97/22.49 and 342.96/171.48, respectively [71]. The ethanol extract, total alkaloid fraction, and pure compounds of *Haplophyllum myrtifolium* Boiss., a medicinal plant endemic in Turkey, were evaluated against *T. vaginalis*. Authors determined the MIC/MLC for each sample, resulting in 200/400, 400/800 and 50/150 µg/mL for ethanol extract, alkaloid extract, and skimmianine after 48 h of incubation, respectively [72]. Ethnopharmacological knowledge drove the trichomonacidal investigation of *Asclepias curassavica* L. However, the ethanolic extract of *A. curassavica* leaves and stem showed poor anti-*T. vaginalis* activity, with an IC_{50} value of 302 µg/mL [73]. Persian traditional medicine contributed to antiparasitic drug research by recommending the use of Rose oil (*Rosa damascena* Mill.) to treat infectious diseases associated with the female genitourinary tract. The hydroalcoholic extract and oil of *R. damascene* showed anti-*T. vaginalis* activity in a dose-related manner, with an IC_{50} of 1.41 and 1.79 mg/mL, respectively [74].

Marketed products used in the culinary tradition, as well as repositioning treatments, also aroused the interest of researchers in the area of the development of new therapeutic alternatives against trichomoniasis. Allium sativum, in commercially available garlic (Tomex®) tablets, was dissolved in distilled water, and the anti-*T. vaginalis* activity was tested. The authors related that the trichomonacidal effect was time- and dose-dependent, where the MIC values were 100 µg/mL in the first 24 h, 50 µg/mL after 48 h, 25 µg/mL after 72 h, and 12.5 µg/mL after 96 h [75]. *Curcuma longa* L. is used for polyphenol curcumin-production, which is widely used in Indian Ayurvedic medicine, food coloring, and several pharmacological processes. The trichomonacidal effect of curcumin after 24 h was observed by growth inhibition with 400 µg/mL against MTZ-resistant and -sensitive isolates, showing an IC_{50} of 105.8 µg/mL and 73.0 µg/mL, respectively [76]. Furthermore, the effect of curcumin on *T. vaginalis* viability was further investigated by another study that found EC_{50} values of 117 µM (24 h) and 173 µM (48 h). The authors related trichomonacidal effects due to the modulation of the enzyme activity and gene expression of pyruvate-ferredoxin oxidoreductase, decreased hydrogenosomal membrane potential, and impacts on the proteolysis of *T. vaginalis* [77]. *Zingiber officinale* Roscoe and its components have been the target of several investigations into their pharmacological properties. After 24 h, ethanol extract presented an IC_{50} of 93.8 µg/mL, and 48 h of incubation at 800 µg/mL was necessary to reduce 100% parasite viability. Moreover, low doses of ginger were able to induce early and late apoptosis in *T. vaginalis* [78]. Ginger, erroneously cited as Ginger officinale, was also used in combination with *Verbascum thapsus* L., which demonstrated the absence of trophozoite growth using 800 µg/mL of alcoholic extract at 48 h of incubation. The IC_{50} value obtained for the combination was 73.8 µg/mL, while the value obtained for MTZ was 0.0326 µg/mL [79]. Phytochemical-rich food-derived evaluation highlights the anti-*T. vaginalis* effect of black tea extract. The theaflavin-rich extract's IC_{50} values were 0.0118%, 0.0173%, and 0.0140% *w/w* against MTZ-sensitive and -resistant and cytoadherent fresh clinical isolates [80]. Cherry tomato was also the target of trichomonacidal research through the peel powder derived from several species. At a concentration of 0.02%, cherry peel powders from organic *Solanum lycopersicum* var. *cerasiforme* (Dunal) D.M. Spooner, G.J. Anderson and R.K. Jansen presented more than 50% activity against *T. vaginalis* [81].

Eicosapentaenoic acid (EPA), also known as omega-3 polyunsaturated fatty acid, was approximately 90% effective at 24 h (with concentrations of 190 µM and 380 µM) against MTZ-sensitive and -resistant *T. vaginalis* isolates, while 100 µM abolished parasite growth at 48 h [82]. Another drug repositioning study with NP-based molecules involved the use of pentamycin against *T. vaginalis*. The authors evaluated the molecule against isolates with distinct levels of susceptibility, from highly sensitive to MTZ-resistant, and described EC_{50} values between 2.36 and 3.62 g/mL after 6 h of incubation [83].

Although the number is smaller, studies using biomolecules with antiparasitic evaluation against *T. vaginalis* were also carried out in an in vivo model of infection. In this context, another antimicrobial peptide explored against *T. vaginalis* infection in mice was isolated from *Epinephelus coioides*. Epinecidin-1 (Epi-1) was able to induce 100% growth inhibition at 62.5 µg/mL and 400 µg, effectively abolishing *T. vaginalis* load in *L. acidophilus*-pre-established mice [84]. Women with recalcitrant cases of trichomoniasis against MTZ or tinidazole were recruited to verify the effect of *Commiphora molmol* Engl. ex Tschirch against the parasite. The oleo-resin extract from *C. molmol* was administered as two capsules (600 mg) for 6 to 8 consecutive days on an empty stomach, followed by the evaluation of trichomoniasis symptoms and microscopy analysis. Among patients with infection resistant to standard treatment and receiving the proposed treatment, the cure rate was 84.6% [85].

Randomized controlled trials were described for *Mentha crispa* L. and *Zataria multiflora Boiss.*, and for probiotic alternative treatment of trichomoniasis. The first consisted of a double-blind and controlled clinical trial consisting of pre-treatment, treatment, and post-treatment phases through use of 24 mg *M. crispa* or 2 g of secnidazole. After treatment, no significant difference was observed between groups, with at least 90% cure rates, showing the effectiveness and safety of *M. crispa* against *T. vaginalis* [86]. In a double-blind clinical trial to assess the effect of vaginal cream containing 0.1% of *Zataria multiflora* or an oral MTZ pill, over seven days, the investigational group was given 5.0 g each night through vaginal application, and the standard group received 250 mg of oral MTZ to use every 12 h for the same period. The authors described that *Z. multiflora* topical treatment had similar effects to oral MTZ, and suggested the use of this NP to eradicate clinical symptoms of trichomoniasis [87].

The use of a combinatory therapy using probiotics and MTZ was recently evaluated in cases of trichomoniasis plus bacterial vaginosis in ninety women, 20–30 years old. This placebo-controlled and double-blind study was performed by the intravaginal administration of 500 mg MTZ and one capsule of probiotic Gynophilus® (*Lactobacillus rhamnosus*), both used two times per day, while the placebo group received only MTZ treatment and a placebo as a substitute for the probiotic. It was related that the new therapy increased the cure rates of trichomoniasis (88.6%) compared to the standard group (42.9%), in addition to reducing the inflammatory response and vaginal pH values [88].

Efforts have been made by researchers in recent years focusing on the search for new molecules of natural origin for the treatment of trichomoniasis. The results, especially in vitro, show great potential for these molecules to be used as new therapeutic approaches. There is still a need for further investigations into the targets of these molecules, as well as the evaluation of the toxicity and efficacy of these NP in in vivo models.

4. Nanotechnology

The topical treatment of human trichomoniasis has attracted the interest of many researchers, since the vaginal route has advantages such as good contact surface and permeability to drugs, ease of administration, and reducing the chance of side effects related to the treatment [89]. However, due to the mucus in the vaginal region, the drug residence time is reduced, leading to inefficient delivery to the site and ineffective treatment [90]. Formulations containing drugs to be topically applied in the vagina must overcome all these challenges, adding to the need for a low propensity to cause genital irritation and systemic toxicity [91]. In addition, the increased biological effect demonstrated by nanoencapsulated molecules in comparison to free compounds has already been described [92].

The authors obtained drug-free chitosan-coated poly(isobutylcyanoacrylate) nanoparticles with diameters in the range of 185–210 nm, and performed the coating with a combination of chitosan and thiolated chitosan. The presence of chitosan in nanoparticle shells was related to strong anti-*T. vaginalis* activity at a concentration of 100 µg/mL. The toxicological evaluation was made in an ex vivo model of porcine mucosal vagina. The demonstration of normal cell architecture without alterations in the stroma through histology images highlighted the absence of toxicity in this model [93]. Thermoresponsive Pluronic® F127 hydrogel was also used to develop another formulation containing nanoparticles loaded with auranofin, previously described as a promising synthetic molecule for trichomonacidal therapy [34][94]. Nanoparticles containing auranofin could inhibit the parasite's growth at dilutions as low as 0.63% (*v*/*v*); however, the final formulation showed an EC_{50} of 22 µM, almost 8-fold less potent than the value obtained for the drug (2.7 µM). Trichomonacidal evaluation was performed in in vivo mice model infected with the parasite responsible for bovine trichomoniasis, *T. foetus*, by the administration of auranofin-loaded nanoparticles embedded in hydrogel for five intravaginal doses (50 µg auranofin/mouse) over three days. All mice showed decreased infection after treatment, while eradication was observed in half of the mice, and it was observed that a single dose was able to cause parasite clearance. An even greater effect was observed with the oral administration of free auranofin. Toxicological analysis demonstrated the absence of a significant influence of hepatic thioredoxin reductase, considering the parasite's target of action [94].

Nanocapsules were also used to develop a gellan gum-based hydrogel containing the active indole-3-carbinol (I3C) for trichomoniasis treatment. The nanoparticle size obtained was 211 nm, and the biological evaluation was carried out by in vitro viability assay, compared with a free compound assay. I3C-loading nanocapsules had an IC_{50} value of 2.09 µg/mL, while the evaluation of the isolated molecule showed an IC_{50} of 3.36 µg/mL, highlighting the advantage of nanoencapsulation to improve the biological effect. The authors used a chorioallantoic membrane method for the irritation potential evaluation to demonstrate its non-irritating character [95]. The success of nanoecapsulation in improving activity against *T. vaginalis* was also demonstrated with nano-liposomal MTZ development. The authors demonstrated, through the analysis of the in vitro trichomonacidal activity of nanoliposomes with a size of 146.8 nm, an IC_{50} value of 15.9 µg/mL after 6 h of incubation, while the free-form presented a higher IC_{50} value (31.51 µg/mL). Still, 12 h was necessary for the nanolipossomal formulation to lyse *T. vaginalis* entirely, while MTZ required 24 h to cause this effect [96].

Obtaining natural products was also the focus of nanotechnological production in the context of trichomoniasis. In this sense, the anti-*T. vaginalis* effect of leaves from *Mikania cordifolia* (L.f.) Willd. (erroneously cited as *Micana cordifolia*) was explored by the development of a nanoemulsion, and compared with MTZ. The effect of nanoemulsion-loaded *M. cordifolia* was evaluated by growth inhibition rate through an in vitro assay, and the results show that a concentration of 1000 ppm after 72 h of incubation has a trichomonacidal ability, as found for MTZ [97]. *Citrullus colocynthis* and *Capparis spinosa* L. also demonstrated anti-*T. vaginalis* activities when evaluated as nanoemulsion. For both, the major effect was observed after 72 h of incubation at 500 ppm, showing growth inhibition rates higher than or equal to those obtained for MTZ [98]. Moreover, the development of nanoparticles from chitosan extracted directly from *Penicillium waksmanii*, *P. aurantiogriseum*, *P. viridicatum*, and *P. citrinum* was described. The authors demonstrated the anti-*T. vaginalis* activity of nano-chitosan, with particles slightly less than 100 nm, presenting an IC_{50} of 11 µg/mL. The nanoencapsulated form of chitosan impaired trophozoite viability up to 99.4% within 48 h of exposure, while the same concentration of chitosan was able to cause a 64.7% mortality rate [99].

The research presented involving the production of nanostructured systems for the treatment of trichomoniasis opens up possibilities for creating more effective, targeted, and safe delivery systems.

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