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Review

Novel Treatment Approaches to Combat Trichomoniasis, a Neglected and Sexually Transmitted Infection Caused by *Trichomonas vaginalis*: Translational Perspectives

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Abstract: The multistep translational science behind new drugs comprehends the entire process through laboratory, clinical, and community observations turned into health interventions. The development of new drug options from discovering targets and leading compounds in basic research for implementing therapeutic guidelines contributes to the emergence of health policies essential for infection control. This review updates the translational research in the scenario of the most common non-viral sexually transmitted infection (STI), trichomoniasis. Paradoxically to its high occurrence, it is considered neglected since notification is not mandatory. It turns into a stable disease with health complications, and receives little emphasis from public health programs to control STI. Although related to curable STIs, the current drugs, metronidazole and tinidazole, present therapeutic failures. The need for new options to treat trichomoniasis is established by basic research studies and patents revealing novel synthetic compounds and natural products presenting anti-*Trichomonas vaginalis* activities, mainly based on in vitro findings. Clinical trials are still focused on new routes of administration for conventional drugs. In addition, nanotechnology approaches are in their infancy, shedding light on potential possibilities for creating more effective, targeted, and safe delivery systems. Overall, the novel proposed approaches need, in addition to pharmaceutical development and efficacy assessments, to ensure that the quality requirements for their use as medicines are met. It is essential to overcome these issues to cross the "Death Valley" of drug discovery and to advance in the translational science criteria in the trichomoniasis drug development field.

Keywords: trichomoniasis; translational science; basic research; natural products; nanotechnology; patents; clinical trials

Citation: Rigo, G.V.; Frank, L.A.; Galego, G.B.; Santos, A.L.S.d.; Tasca, T. Novel Treatment Approaches to Combat Trichomoniasis, a Neglected and Sexually Transmitted Infection Caused by *Trichomonas vaginalis*: Translational Perspectives. *Venereology* **2022**, *1*, 47–80. https://doi.org/10.3390/venereology1010005

Academic Editor: Sylvia Bruisten

Received: 30 November 2021
Accepted: 27 January 2022
Published: 29 January 2022

1. Introduction

The science behind new drugs permeates the entire development process, from discovering targets and leading compounds in basic research to implementing therapeutic guidelines and the emergence of health policies. This multistep translational process is an approach of the National Center for Advancing Translational Sciences (NCATS), from the National Institutes of Health (NIH-USA), performed through laboratory, clinical, and community observations turned into health interventions. In this context, the difference between implementing translational research and translational science concepts is important. The first focuses on specific targets and diseases, while the last aims to elucidate

general operative principles [1]. Despite efforts to increase knowledge and funding centers as the driving force of the translational process, Austin [2] describes that the time required for laboratory discoveries to reach the population is estimated to be more than ten years, with about a 1% rate of success. The main setbacks of this process are the high cost for new drug development, the inefficiency of clinical trials and the slow spread of new treatment alternatives to the population [2]. Furthermore, there is an important gap between fundamental research and the development of medical products, strongly impacting the knowledge creation stage of translational research. This is described as "Death Valley", where some research does not progress to clinical stages, presenting failures to qualify and industrialize as important impeding components [3,4]. In this scenario, neglected and widely disseminated infections present a major challenge in translational research.

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 [5,6]. 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 [6]. 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 [7,8]. 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 [9].

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 [10]. The complementary intravaginal treatment with boric acid, paromomycin sulfate, povidone iodine, and furazolidone appears to show some efficacy, but lesser than approved drugs [11]. 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 [12].

Translation research on the anti-*T. vaginalis* drug development pipeline aims to transpose the laboratory microenvironment to more complex systems involving humans. In recent years, several studies have demonstrated the potential of synthetic molecules and biomolecules, in free or in nanoencapsulated forms, as promising alternatives for treating trichomoniasis. However, practical interventions in public health to increase cure rates, with the use of molecules capable of escaping resistance mechanisms, are required. In this context, this review highlights new approaches to trichomoniasis treatment, focusing on laboratory experimentation, patent elaboration, and clinical trials. In addition, we investigated the time spent for scientific research on treating trichomoniasis going forward, and the reasons to delay translational research in the scenario of the most common but overlooked non-viral STI.

2. Methodology

For this research, scientific databases such as Pubmed (https://pubmed.ncbi.nlm.nih.gov), Espacenet (https://worldwide.espacenet.com) and Clinical Trials (https://clinicaltrials.gov) were considered, following the criteria shown in the Figure 1. All information found comes from the last ten years of research from the bench to the patient.

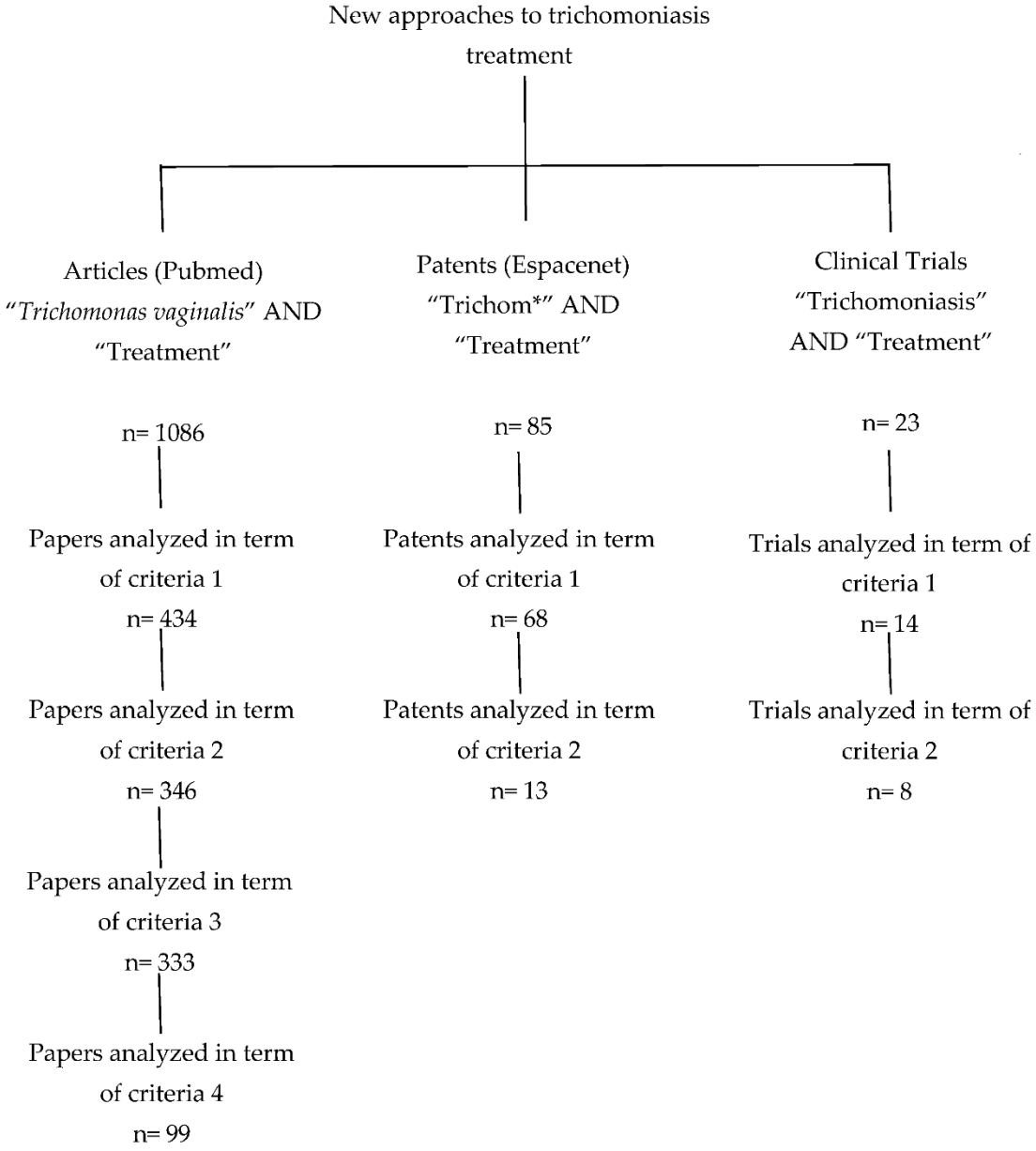

Figure 1. Flowchart of the research carried out for the analysis of articles (Pubmed), patents (Espacenet) and clinical trials. For Pubmed: criteria 1—studies carried out in the last 10 years (from 2021 to 2011); criteria 2—exclusion of reviews and articles with no access; criteria 3—only studies in English; criteria 4—only experimental papers with anti-*T. vaginalis* activity demonstration/treatment-related studies for trichomoniasis. For Espacenet and clinical trials: criteria 1—treatment-related studies for trichomoniasis; criteria 2—studies carried out in the last 10 years (from 2011).

3. Results and Discussion

The literature analysis obtained from online databases allowed for the compilation of new approaches for trichomoniasis treatment (2011–2021), involving synthetic compounds, natural products, and nanotechnology against the parasite *T. vaginalis*. Figure 2 shows the worldwide distribution of the scientific efforts to combat this STI, as used in this review. Basic science represented by article publications takes the lead, demonstrating the research concern in prospecting new and old molecules useful for their anti-*T. vaginalis* activity. However, the major challenge of developing of new trichomonacidal drugs is represented by a drastic reduction in patent production and clinical trials development, restricted to

certain locations. In this review, we faced heterogeneity in presenting the data reflecting the absence of validation in methodology assays, and the analysis of anti-*T. vaginalis* activity. This limitation hinders a comparison of results among different groups using distinct isolates, incubation times, and viability assays.

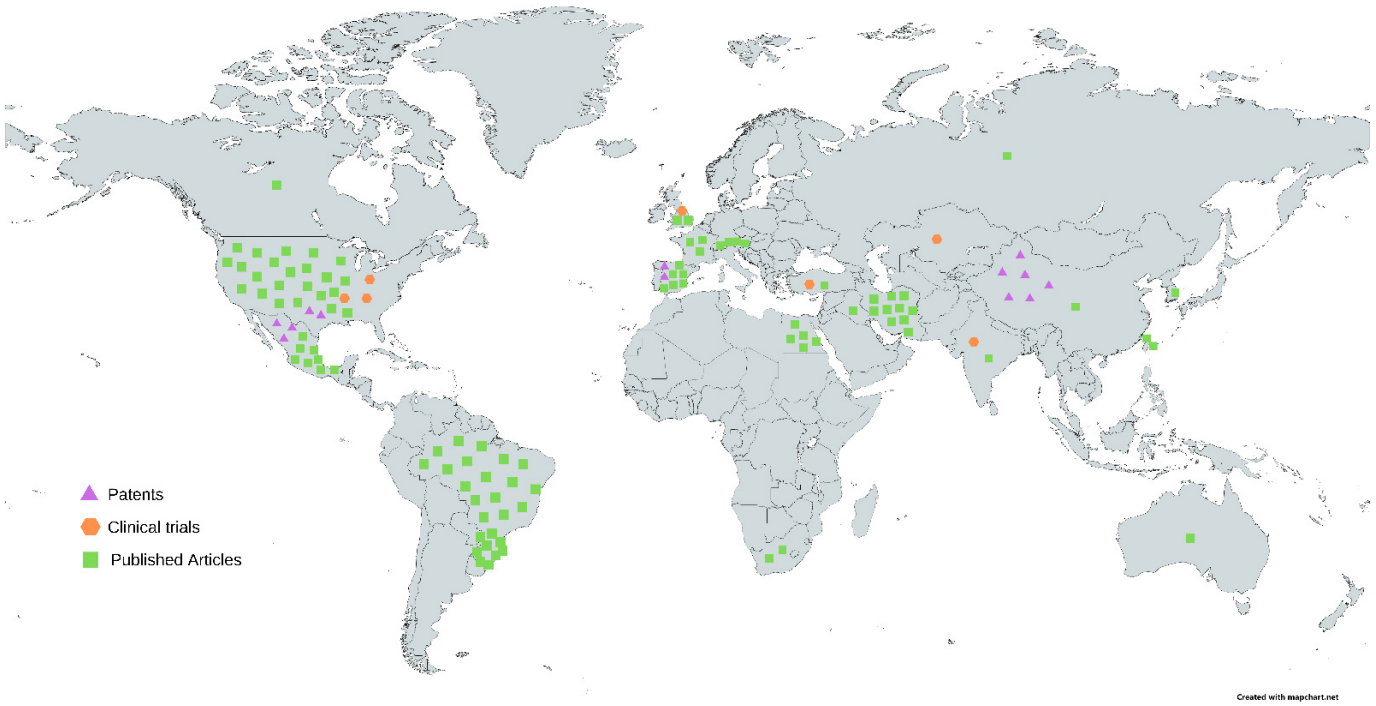

Figure 2. Worldwide distribution planisphere of new approaches developed for treating trichomoniasis reviewed in this study. Published articles (green squares), clinical trials (orange hexagon), and patents (purple triangle).

3.1. New Scientific Approaches from Basic Research (Articles)

In the drug discovery process, the contribution of laboratory benches is substantial, through in silico and in vitro screening of synthetic compounds and molecules derived from natural products, known as biomolecules, with anti-*T. vaginalis* activities. Promising candidates can exhibit effectiveness at lower doses than the reference drugs, and the elucidation of biological targets allows for the search for molecules that escape from known resistance pathways. Considering that *T. vaginalis* occurs in the human genitourinary tract, in vivo testing using animal models for human trichomoniasis is still incipient. In this sense, NCATS has developed drug discovery, development, and deployment maps to guide the different process stages, and highlighted substantial differences in small molecules and biologic products related to therapeutic candidate identification and optimization [13,14]. In the last decade, anti-*T. vaginalis* basic research increased considerably, and new approaches from the laboratory bench were summarized in this topic, through the presentation of promising molecules of natural and synthetic origins, as well as the use of nanotechnology involved in the treatment of trichomoniasis (Table 1).

Table 1. Basic research on promising molecules for the treatment of trichomoniasis of natural and synthetic origin, as well as nanotechnology approaches.

Most active Compounds	Dose	Testing Method	Pharmaceutical Form	Reference
Synthetic Compounds				
(Tri-*n*-ethylphosphine)gold(I) chloride (4)	pEC_{50}: 6.06 μM (24 h)	in vitro (*T. vaginalis*), in vivo (*T. foetus*)	Solution	[15]
(Tri-*n*-methylphosphine)-gold(I) chloride (10)	pEC_{50}: 5.84 μM (24 h)	in vitro (*T. vaginalis*), in vivo (*T. foetus*)	Solution	[15]
1,10-phenanthroline-5,6-dione-based metallodrugs (Copper-phendione)	MIC: 8.84 μM (24 h) IC_{50}: 0.87 μM (24 h)	in vitro	Solution	[16]
1,3-dioxolanes that contain tellurium (PTeDOX 01)	MIC: 90 μM and IC_{50}: 60 μM (24 h)	in vitro	Solution	[17]
1,5-bis(2-chlorophenyl)penta-1,4-dien-3-one (**3e**)	MIC/IC_{50}: 90 μM/50 μM (24 h)	in vitro	Solution	[18]
1,5-diphenylpenta-1,4-dien-3-one (**3a**)	MIC/IC_{50}: 80 μM/50 μM (24 h)	in vitro	Solution	[18]
2-Benzyl-3-(3-hydroxypropoxy)-5-nitro-2H-indazole	IC_{50}: 7.25 and 9.11 μM (24 h) (sensitive and resistant strains)	in vitro	Solution	[19]
2,2′-[α,ω-propanediylbis(oxy-1,3-phenylene)]bis-1H-benzimidazole	MIC: 9.0 μM (48 h)	in vitro, in vivo	Solution	[20]
2,4-diamine-quinazoline derivative (PH100)	Clinical isolate: MIC/IC_{50} 80 μM/14.8 μM. (24 h) long-term-grown: MIC/IC_{50} 90 μM/50 μM (24 h)	in vitro	Solution	[21]
2,6-bis(2-chlorobenzylidene)cyclohexanone (5e)	MIC/IC_{50}: 200 μM/70 μM (24 h)	in vitro	Solution	[18]
2′-Hydroxychalcones (3c)	MIC: 100 μM (24 h) IC_{50}: 50.64 μM (24 h)	in silico, in vitro	Solution	[22]
3-(aminoalkoxy)indazoles (27)	IC_{50}: 5.6 and 8.5 μM (24 h) (sensitive and resistant strains)	in vitro	Solution	[23]
3-(biphenyl-4-yl)-3-hydroxyquinuclidine (BPQ-OH)	IC_{50}: 46 μM (24 h)	in vitro	Solution	[24]
3-(ω-aminoalkoxy)-1-benzyl-5-nitroindazoles (6)	IC_{50}: 19.2 and 1.3 μM (sensitive and resistant strains)	in vitro	Solution	[25]
3-(ω-aminoalkoxy)-1-benzyl-5-nitroindazoles (10)	IC_{50}: 2.5 and 0.5 μM (sensitive and resistant strains)	in vitro	Solution	[25]
3,3′-{[4-(4-morpholinyl)phenyl] methylene} bis (4-hydroxy-2H-chromen-2-one) (A4)	IC_{50}: 47 μM (24 h)	in silico, in vitro	Solution	[26]
3-alkoxy-5-nitroindazoles derivatives	GI: 40% (1.0 μg/mL) (24 h)	in silico, in vitro	Solution	[27]

Table 1. *Cont.*

Most active Compounds	Dose	Testing Method	Pharmaceutical Form	Reference
3′-aminochalcone (3)	IC_{50}: 29 μM (24 h)	in vitro	Solution	[28]
3-oxime-urs-12-en-28-oic-ursolic acid (9)	MIC: 25 μM (24 h)	in vitro	Solution	[29]
5-Bromo-1-[3-(2,3-dioxo-2,3-dihydro-indol-1-yl)propyl]-1*H*-indole-2,3-dione (4t)	IC_{50}: 3.72 μM (24 h)	in vitro	Solution	[30]
5-Chloro-6-ethoxy-2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}-1-methyl-1*H*-benzimidazole (51)	IC_{50}: 0.0698 μM	in vitro	Solution	[31]
7-deaza,7-(3,4-dichlorophenyl)adenosine (FH3147)	EC_{50}: 0.029 μM (24 h)	in vitro (*T. vaginalis*), in vivo (*T. foetus*)	Solution	[32]
7-Nitro-4-(3-piperidinopropyl)quinoxalin-2-one	IC_{50}: 18.26 μM (24 h)	in vitro, in vivo	Solution	[33]
9-(2-deoxy-2-fluoro-β,D-arabinofuranosyl)adenine	IC_{50}: 0.09 μM (24 h)	in vitro	Solution	[34]
A5 ($C_{22}H_{26}N_4O_4S_2$)	IC_{50}: 105.2 μM (24 h)	in silico, in vitro	Solution	[35]
Auranofin	IC_{50}: 0.7–2.5 μM and MLC: 2.0–6.0 μM (24 h)	in vitro *(T. vaginalis)*, in vivo (*T. foetus)*	Solution	[36]
B3 ($C_{16}H_{15}N_5O_4S_2$)	IC_{50}: 66.6 μM (24 h)	in silico, in vitro	Solution	[35]
Betulinic acid derivative (4)	MIC: 25–50 μM (24 h)	in vitro	Solution	[37]
Boric acid	MLC: 0.3–0.6%	in vitro	Solution	[38]
C-131	IC_{50}: 0.033 μM	in vitro	Solution	[39]
C-120	IC_{50}: 0.173 μM	in vitro	Solution	[39]
C4 ($C_{14}H_{28}N_6O_2S_2$)	IC_{50}: 98.3 μM (24 h)	in silico, in vitro	Solution	[35]
Chlorinated metronidazole	IC_{50}: 0.006 and 0.24 μM (48 h) (sensitive and resistant strains)	in vitro	Solution	[40]
Cinnamoyl-Oxaborole Amides: (E)-*N*-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(4-nitrophenyl)acrylamide (5c)	IC_{50}: 10.2 μM (24 h)	in vitro	Solution	[41]
Diamine derivative (4):	MIC: 70 μM (24 h)	in vitro	Solution	[42]
Disulfiram	IC_{50} (μM) value (aerobic/anaerobic): 0.06/0.09 for MTZ-sensitive and 0.10/1.52 MTZ-resistant (48 h)	in vitro	Solution	[43]
Fumagillin	IC_{50}: 0.26 μM (48 h)	in silico, in vitro	Solution	[44]
Furanyl *N*-acylhydrazone derivatives (PFUR 4a)	IC_{50}: 1.69 μM (24 h)	in silico, in vitro	Solution	[45]
Furanyl *N*-acylhydrazone derivatives (PFUR 4b)	IC_{50}: 1.98 μM (24 h)	in silico, in vitro	Solution	[45]
Lansoprazole	IC_{50}: 0.12 μM	in vitro	Solution	[46]

Table 1. *Cont.*

Most active Compounds	Dose	Testing Method	Pharmaceutical Form	Reference
Metronidazole	MTZ (0.7 wt. %) combined with pluronic® F127 (20 wt. %) and chitosan (1 wt. %)	in vitro	Hydrogel	[47]
Metronidazole, tinidazole and boric acid	500 mg MTZ every 8 h/7 day + tinidazole 2 g + 600 mg boric acid	case reports	Intravenous (MTZ), liquid (tinidazole), and intra-vaginal (boric acid)	[48]
Metronidazole	500 mg MTZ (one week)	case report	Intravenous and vaginal ge	[49]
Metronidazole	2 g (single-dose group) or 500 mg twice daily for 7 days (7-day-dose group).	randomized controlled trial	Oral	[50]
Metronidazole	2 g (single-dose) versus 500 mg twice daily for 7-days (multi-dose)	clinical trial	Oral	[51]
Metronidazole and Miconazole	MTZ 750 mg plus miconazole 200 mg (5 consecutive nights each month for 12 months)	Randomized Controlled Trial	Vaginal suppositories	[50]
Metronidazole/miconazole	MTZ 750 mg/miconazole nitrate 200 mg (once or twice a day)	randomized controlled trial	vaginal suppository	[52]
Metronidazol/RAMEB and Metronidazol/CRYSMEB	0.01 to 10 µg/mL (24 h)	in vitro	Solution	[53]
Miltefosine	IC_{50}: 14.5 µM (24 h)	in vitro	Solution	[54]
Nitazoxanide	MLC: 50 µg/mL (MTZ-resistant) and 6.0 (MTZ-sensitive) (24 h)	in vitro	Dilution	[55]
Nithiamide	IC_{50} (µM) value (aerobic | anaerobic) of 1.33 | 0.78 for MTZ-sensitive and 5.88 | 1.51 MTZ-resistant (48 h)	in vitro	Solution	[43]
Nitroimidazole carboxamides	EC_{50} = 0.6–1.4 µM	in vitro	Solution	[56]
N-chlorotaurine (NCT) in combination with NH_4Cl	5.5 mM (0.1%) NCT plus 19 mM (0.1%) NH_4Cl (5 min)	in vitro	Solution	[57]
Octenidine dihydrochloride with phe-noxyethanol	EC_{50}: 0.68–2.11 µg/mL (30 min)	in vitro	Dilution	[58]
Omeprazole	IC_{50}: 0.1216 µM	in vitro	Solution	[46]
Pantoprazole	IC_{50}: 0.0756 µM	in vitro	Solution	[46]
Paromomycin and tinidazole	5.0 g of a 5.0% (paromomycin) with concomitant oral tinidazole 1.0 g 3 times daily for 14 days	case reports	intravaginal cream (paromomycin) and tablet (tinidazole)	[20]
Photodynamic therapy: methylene blue and light-emitting diode	68.1 J/cm^2 (35.6 s.)	in vivo	fiber-optic tip 2 mm in diameter to the LED device	[59]
Rabeprazole	IC_{50}: 0.1057 µM	in vitro	Solution	[46]

Table 1. *Cont.*

Most active Compounds	Dose	Testing Method	Pharmaceutical Form	Reference
Secnidazole	2 g	clinical trial	Oral Granules	[60]
Secnidazole	MLC: 1.6 μg/mL	in vitro	Solution	[61]
Tetracycline	Cytotoxic effect: 700 μg/mL (4 h)	in vitro	Dilution	[62]
Tinidazole	3.3–1000 mg	case report	Oral	[63]
Tinidazole and Paromomycin Combination	oral tinidazole (1 g, 3 times daily) and 4 g of 6.25% intravaginal paromomycin	case report	Cream (paromomycin) and tablet (Tinidazole)	[64]
Zinc–clotrimazole complex (Zn(CTZ)2(Ac)2)	IC_{50}: 4.9 μM (48 h)	in vitro	Solution	[65]
Zinc sulfate	1% (14–28 days)	case report	Douche	[66]
Natural Products				
Amomum tsao-ko Crevost and Lemarié (essential oil and geraniol)	MLC/IC_{50} (μg/mL) of 44.97/22.49 and 342.96/171.48 (48 h)	in vitro	Solution	[67]
Asclepias curassavica L. (Apocynaceae) (ethanol extract)	IC_{50}: 302 μg/mL (24 h)	in vitro	Solution	[68]
Basidiomycete *Amauroderma camerarium* (Amaurocine)	MIC: 4.56 μM (24 h)	in vitro	Solution	[69]
Bidens Pilosa L	MIC: 1.0 mg/mL (24 h)	in vitro	Solution	[70]
Combination *Verbascum thapsus* L. and *Zingiber officinale* Roscoe (erroneously cited as *Ginger officinale*) (alcoholic extract)	IC_{50}: 73.80 μg/mL	in vitro	Solution	[71]
Commiphora molmol Engl. ex Tschirch (Mirazid)	two capsules (600 mg) for 6 to 8 consecutive days	humans	Capsules	[72]
Curcuma longa L. (Curcumin)	EC_{50}: 73.0–105.8 μg/mL	in vitro	Solution	[73]
Curcumin	IC_{50}: 117 ± 7 μM (24 h) and 173 ± 15 μM (48 h)	in vitro	Solution	[74]
Eicosapentaenoic Acid	100 μM (48 h)	in vitro	Solution	[75]
Epinecidin-1 (synthetic fish antimicrobial peptide)	Growth inhibition: 62.5 μg/mL (180 min)	in vivo and in vitro	Solution	[76]
Eucalyptus camaldulensis Dehnh.	60 μg (72 h)	in vitro	Solution	[77]
Eucalyptus camaldulensis Dehnh. (Ethyl acetate fraction)	GI: 12.5 mg/mL (24 h)	in vitro	Solution	[78]
Eucalyptus camaldulensis Dehnh. (phenolic extract), *Viola odorata* L. (phenolic extract), and *Mentha piperita* L. (hydroalcoholic extracts)	100% *T. vaginalis* growth inhibition (24 h): 2.5 mg *E. camaldulensis*, 0.06 mg *V. odorata*, and 1.0 mg *M. piperita*/1.0 g of cream	in vitro	Vaginal creams	[79]
garlic-based product (Tomex®)	MIC: 100 μg/mL (24 h), 50 μg/mL (48 h), 25 μg/mL (72 h), and 12.5 μg/mL (96 h)	in vitro	Solution	[80]
Haplophyllum myrtifolium Boiss. (ethanol extract, alkaloid extract, and skimmianine)	MIC/MLC (μg/mL): 200/400, 400/800, and 50/150 (48 h)	in vitro	Solution	[81]

Table 1. *Cont.*

Most active Compounds	Dose	Testing Method	Pharmaceutical Form	Reference
Hypericum L. spp. (phloroglucinol derivative isoaustrobrasilol B)	IC_{50}: 38 µM (24 h)	in vitro	Solution	[82]
Hypocrea lixii (F02) and *Penicillium citrinum* (F40)	MIC: 2.5 mg/mL (24 h)	in vitro	Solution	[83]
Kalanchoe daigremontiana Raym.-Hamet and H. Perrier (flavonoid quercetin and methanol extract)	IC_{50}: 21.17 µg/mL and 105.27 µg/mL, respectively	in vitro	Solution	[84]
Manilkara rufula (Miq.) H.J.Lam (H100: enriched saponin fraction)	MIC: 0.5–1.0 mg/mL (24 h)	in vitro	Solution	[85]
Mentha crispa L. (Giamebil®, Hebron Pharmaceutical Industry, Brazil)	24 mg	randomized controlled trial	Tablets	[86]
Morinda panamensis Seem. (anthraquinone lucidin-ω-isopropyl ether)	IC_{50}: 1.32 µg/mL (48 h)	in vitro	Solution	[87]
Ozoroa engleri R. Fern. and A. Fern	MIC: 1 mg/mL (24 h)	in vitro	Solution	[70]
Pentamycin	EC_{50}: 2.36– 3.62 g/mL (6 h)	in vitro	Solution	[88]
Phaseolus vulgaris L. (lecitin) and *Nigella sativa* L. (oil)	500 µg/mL for both	in vitro	Solution	[89]
Pistacia lentiscus L. mastic and *Ocimum basilicum* L. oil	MIC: 15 mg/mL and 30 µg/mL (24 h)	in vitro	Solution	[90]
Polygala decumbens A.W. Benn.	MIC: 1.56 mg/mL (24 h)	in vitro	Solution	[91]
Probiotic Gynophilus® and metronidazole	MTZ at 500 mg twice a day and 1 capsule of probiotic twice a day	randomized, placebo-controlled, double-blind study	Vaginal capsule (probiotic) and oral (MTZ)	[92]
ProProphenin 2 peptide	LD_{50}: 47.66 µM (24 h)	in vitro	Solution	[93]
Pterocaulon balansae Chodat (Coumarins from dry hydroethanolic extract)	MIC: 30 µg/mL and IC_{50}: 3.2 µg/mL (24 h)	in vitro	Solution	[94]
Quillaja saponaria Molina (saponins)	MIC: 0.025%	in vitro	Solution	[95]
Rosa damascena Mill. (Oil and Hydroalcoholic extract)	IC_{50}: 1.79 and 1.41 mg/mL respectively (24 h)	in vitro	Solution	[96]
Sarcophyte sanguinea Sparrm.	MIC: 1 mg/mL (24 h)	in vitro	Solution	[70]
Solanum lycopersicum var. *cerasiforme* (Dunal) D.M. Spooner, G.J. Anderson and R.K. Jansen	GI: 0.02% (24 h)	in vitro	Solution	[97]
Syzygium cordatum Hochst. ex Krauss	MIC: 1 mg/mL (24 h)	in vitro	Solution	[70]
Tabernaemontana elegans Stapf	MIC: 1 mg/mL (24 h)	in vitro	Solution	[70]
Theaflavin-rich black tea extract	IC_{50}: 0.0118–0.0173% *w*/*w* (24 h)	in vitro	Solution	[98]
Ursolic acid	MIC: 50–12.5 µM (24 h)	in vitro	Solution	[99]
Verbena L. sp. and *Campomanesia xanthocarpa* O. Berg	MIC value of 4.0 mg/mL	in vitro	Solution	[100]
Zataria multiflora Boiss.	0.1%/7 days	randomized controlled trial	Vaginal creams	[101]

Table 1. *Cont.*

Most active Compounds	Dose	Testing Method	Pharmaceutical Form	Reference
Zingiber officinale Roscoe (Ginger-alcoholic extract)	IC_{50}: 93.8 µg/mL (24 h) GI: 800 µg/mL (48 h)	in vitro	Solution	[102]
Nanotechnology				
Auranofin-loaded nanoparticles	EC_{50} = 22 µM (24 h)	in vitro (*T. vag*) and in vivo (*T. foetus*)	Hydrogel	[103]
Drug-free chitosan coated poly(isobutylcyanoacrylate) nanoparticles	100 µg/mL (24 h)	in vitro	Hydrogel	[104]
Nanocapsules containg indole-3-carbinol	IC_{50} = 2.09 µg/mL (24 h)	in vitro	Gellan gum-based hydrogel	[105]
Nano-chitosan	IC_{50}: 11 µg/mL	in vitro	Suspension	[106]
Nano-emulsion of *Capparis spinosa* L.	GI: 500 ppm (72 h)	in vitro	Suspension	[107]
Nano-emulsion of *Citrullus colocynthis* (L.) Schrad.	GI: 500 ppm (72 h)	in vitro	Suspension	[107]
Nano-emulsion of *Micana Mikania cordifolia* (L.f.) Willd. (erroneously cited as *Micana cordifolia*)	1000 ppm (72 h)	in vitro	Suspension	[108]
Nano-liposomal metronidazole	IC_{50}: 15.90 µg/mL (6 h)	in vitro	Suspension	[109]

3.1.1. Articles: Synthetic Compounds

In this topic, 99 studies were discussed following the evaluation of synthetic molecules as an alternative for treating trichomoniasis, including new compounds, synthetic derivatives from natural products and repositioned drugs. In vitro and in silico assays, for new synthetic compounds with anti-*T. vaginalis* activity, are the first steps in searching for alternative therapies through screening compound libraries or by guided synthesis of pathway inhibitors. In this sense, 3-(biphenyl-4-yl)-3-hydroxyquinuclidine, an arylquinuclidine derivative, presented an IC_{50} (concentration capable of inhibiting 50% of trophozoite viability) value of 46 µM, and due to the low cytotoxicity observed, the authors suggested its use as a lead compound for the development of new derivatives [24]. The anti-*T. vaginalis* activities of three 1,3-dioxolanes containing tellurium-based compounds were investigated, and one derivative (PTeDOX 01) successfully killed 100% of the trophozoites, showing MIC and IC_{50} values of 90 and 60 µM, respectively [17]. The synthesis of N-alkyl-tethered C-5 functionalized bis-isatins allowed wide antimicrobial activity, and 5-bromo-1-[3-(2,3-dioxo-2,3-dihydro-indol-1-yl)propyl]-1*H*-indole-2,3-dione (compound 4t) exhibited better activity against *T. vaginalis*, with an IC_{50} value of 3.72 µM [30]. Novel cinnamoyl-oxaborole amides were synthesized and evaluated against this protozoan. The most potent derivative in that study was (*E*)-*N*-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(4-nitrophenyl)acrylamide (5c), with an IC_{50} value of 10.2 µM [41]. Chalcones have been the target of research in drug development against *T. vaginalis*, through the synthesis of derivatives with different chemical groups/radicals. The effect of 3′-aminochalcone was investigated for trichomonacidal activity, with an IC_{50} of 29 µM [28]. Another chalcone derivative, 3c, described as (*E*)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one, showed an MIC of 100 µM in 12 h of incubation and an IC_{50} value of 50.64 µM. When 3c was associated (12.5 µM) with MTZ at 40 µM, it demonstrated 95.31% activity against *T. vaginalis* at 24 h. Moreover, in silico analysis showed enzyme inhibition of methionine gamma-lyase, lactate dehydrogenase, and purine nucleoside phosphorylase from the parasite [22]. Another compound, 2,4-diamine-quinazoline derivative (PH100), was evaluated, and demonstrated anti-*T. vaginalis* activity and synergistic interaction with MTZ against different isolates. IC_{50} values ranged between 14.8 and 50 µM, and MIC values were

80.0–90.0 μM, for a fresh clinical isolate and a long-term-grown ATCC strain, respectively, revealing the effect on function and expression of several trophozoite peptidases related to apoptosis cell death [21]. The in vitro evaluation of *N*-chlorotaurine (NCT) demonstrated activity against *T. vaginalis*, of which 5.5 mM NCT (1%) led to the death of 100% parasites in 15 min. This effect was increased by the addition of 19 mM (0.1%) NH_4Cl, which was able to oxidize intracellular proteins more quickly and lead to trophozoite death in just 5 min [57].

In addition to the activity against *T. vaginalis*, some investigations have elucidated the pathways involved in this pharmacological effect. The nucleoside analogue compound 9-(2-deoxy-2-fluoro-β,D-arabino-furanosyl)adenine, a potent inhibitor of the enzyme adenosylhomocysteine hydrolase, showed better in vitro activity than MTZ, with IC_{50} at 0.09 μM, while the IC_{50} for MTZ was 0.72 μM [34]. Polyamine metabolism was targeted for compound development, with the investigation of the anti-*T. vaginalis* activity of *N*-alkylated diamines and amino alcohols. Diamine 4 presented a MIC value of 70 μM and caused trophozoites death by competition with the spermine transporter [42]. Through in silico analysis, thioredoxin reductase and methionine gamma-lyase were described as targets of the two furanyl *N*-acylhydrazone derivatives (PFUR) **4a** and **4b**, presenting IC_{50} values of 1.69 μM and 1.98 μM, respectively [45]. After the high-throughput virtual molecular docking of the molecule screening library ChemBridge, the protein triosephosphate isomerase was defined as the target of three compounds, denominated as A5 ($C_{16}H_{26}N_4O_4S_2$), B3 ($C_{16}H_{15}N_5O_4S_2$), and C4 ($C_{14}H_{28}N_6O_2S_2$), with IC_{50} values (μM) of 105.2, 66.6, and 98.3, respectively [35]. Another potential drug that interferes in triosephosphate isomerase function was identified by in silico analysis as 3,3′-{[4-(4-morpholinyl)phenyl]methylene}bis(4-hydroxy-2*H*-chromen-2-one (A4), presenting an IC_{50} of 47 μM [26].

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 [37]. 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 [29]. 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 [16]. 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 [18]. 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 [44].

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 [54]. The topical use of boric acid is already described in the STI guidelines as an alternative to treat diseases in the female genital tract [10]. 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 [38]. 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 [46].

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 [62]. 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 [58]. 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 [55]. 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 [61]. 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 [65]. 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 [66]. 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 [43].

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 [47]. 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 [53]. 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) [56]. 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 [40]. 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. Among the most active compounds described in that study, we highlight the potent action of C-131 against the MTZ-sensitive isolate (IC_{50}: 0.033 µM), and C-120 with an IC_{50} value of 0.173 µM against the MTZ-resistant isolate [39]. Still, the

development of new compounds derived from benzimidazole is underway with powerful molecules against *T. vaginalis*. 2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}-1*H*-benzimidazole synthesized derivatives demonstrated remarkable trichomonacidal activity in the nanomolar range, with IC_{50} values lower than MTZ, including derivative **51** (5-Chloro-6-ethoxy-2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}-1-methyl-1*H*-benzimidazole), which presented the lowest value (IC_{50}: 0.0698 μM) [31].

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) [27]. 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 [19]. 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 [23]. Recently, Ibáñez-Escribano et al. [25] 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 [33].

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 [110]. 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 [36]. This approach was also used by Natto [32] and Miyamoto [15] 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* [32]. 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**) [15]. 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 [59]. 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 [20].

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 [48,49,63,64]. 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 [111]. 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) [112]. 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 [52]. 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 [113].

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) [50]. The efficacy related to a single oral dose of secnidazole was demonstrated (NCT03935217), compared to the placebo group, with increased microbiological cure [60] 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 [51].

The data collected from synthetic product analysis allow us to identify that most studies involve repositioning existing drugs and changing therapeutic regimens.

3.1.2. Articles: 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 [114]. 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 [115]. 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 [116]. 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 [90]. *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 [89]. 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 [87]. 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 [70]. 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 [82]. 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 [91]. *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 [99]. 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 [85].

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* [95]. 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 [84]. 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 [94].

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* [83]. 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 [69]. 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 [93].

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 [77]. 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 [78]. 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 [79]. 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 [100]. 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 [67]. 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 [81]. 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 [68]. 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 [96].

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 [80]. *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 [73]. 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* [74]. *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* [102]. 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 [71]. 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 [98]. 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* [97]. 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 [75]. 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 [88].

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 [76]. 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% [72].

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 [101].

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 [92].

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.

3.1.3. Articles: 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 [117]. 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 [118]. 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 [119]. In addition, the increased biological effect demonstrated by nanoencapsulated molecules in comparison to free compounds has already been described [120]. Among the main issues, we can highlight modulation caused by cell interaction through increased uptake, and efficient intracellular release by mechanisms of enzymatic degradation and oxidation reduction, as well as amelioration in chemical stability by preventing the appearance of degradation products, improving the bioavailability of drugs and reducing adverse effects [118]. In this sense, nanotechnology has enabled the emergence of a brand new horizon of trichomoniasis treatment.

The first study found in our evaluation to use nanotechnology as a tool for the development of new alternatives against trichomoniasis explored the potential of drug-free mucoadhesive nanoparticles in thermosensitive Pluronic® F127 hydrogel added to the vaginally applied formulation. 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 [104]. 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 [36,103]. 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 [103].

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 [105]. 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 [109].

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 [108]. *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 [107]. 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 [106].

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.

3.2. Technological Prospecting: Patent Searching and Screening

It was possible to find twelve patents from the last decade that proposed some solution or technology for treating trichomoniasis (Table 2). Regarding patent applicants, China leads the way (n = 6), followed by Mexico (3), USA (2) and Spain (2). China has a highly representative patent filing due to its high technological and scientific budget and strong innovation in producing methods to treat diseases (Figure 2). Moreover, technology-based companies (including startups) in healthcare or biotechnology are highly concentrated in China and the USA, who are world leaders in launching new products with applicability in the sectors of treatment and molecular biology.

Table 2. Solution or technology for treating trichomoniasis proposed by patents.

Active	Dose	Testing Method	Pharmaceutical Form	Inventor (Patent Applicants)	Identification	Reference
Synthetic Drugs						
1,6-bis (N1-*p*-chlorophenyl-N5-biguanidino) hexane	Aqueous acetate solution: 1%, 0.1% and 0.01% (*m*/*v*) Purified aqueous gluconate: 1%, 0.1% and 0.05% (*m*/*v*).	Patient	Lotion	DUAN JINGCHAO	CN106667983A	[121]
3 amine derivatives (1-aminoalquil)indazolinonas, 3-(aminoalcoxi)indazoles and 3-(alquilamino)indazoles]).	DMSO Solution: 300 µM (maximum dose)	In vitro	Solution	ESCARIO GARCIA-TREVIJANO, JOSÉ ANTONIO; GOMEZ BARRIO, ALICIA; NOGAL RUIZ, JUAN JOSÉ; FONSECA BERZAL, CRISTINA ROSA; IBANEZ ESCRIBANO, ALEXANDRA; ARAN REDO, VICENTE JESÚS; DARDONVILLE, CHRISTOPHE; VELA ORTEGA, NEREA; SIFONTES RODRIGUEZ, SERGIO; MENESES MARCEL, ALFREDO IRENALDO	ES2653674B2	[122]
3,3′-{[4-(4-morpholinyl) phenyl] methylene} bis (4-hydroxy-2*H*-chromen-2-one) or hereafter referred to indistinctly as compound **A4**, and derivatives	100 µM	In vitro	Solution	BENITEZ CARDOZA CLAUDIA GUADALUPE	WO2018065807A1	[123]
3,3′-{[4-(4-morpholinyl) phenyl] methylene} bis (4-hydroxy-2*H*-chromen-2-one) or hereafter referred to indistinctly as compound **A4** and 5,5′-[(4-nitrophenyl) methylene] bis (6-hydroxy-2-mercapto-3-methyl-4 (3*H*) -pyrimidinone or hereafter referred to indistinctly as compound **D4**	IC_{50} (1:3 ratio of A4 + D4 respectively): 48 µM (12 µM A4 + 36 µM D4).	In vitro	Solution	BENITEZ CARDOZA CLAUDIA GUADALUPE	WO2018065809A1	[124]
5,5′-[(4-nitrophenyl) methylene] bis (6-hydroxy-2-mercapto-3-methyl-4 (3*H*)-pyrimidinone or hereafter referred to indistinctly as compound **D4**	Cl_{50}: 153 µM.	In vitro	Solution	VIQUE SÁNCHEZ JOSÉ LUIS-BENITEZ CARDOZA CLAUDIA	WO2018065808A1	[125]
Diindolylmethane compounds-related indoles	100–200 mg orally once or twice a day for 1–2 weeks		Oral	ZELIGS MICHAEL	US2010055201A1	[126]
Secnidazole	2 g as a single dose	Patient	Microgranule	PENTIKIS HELEN S	US2020289470A1	[121]
three families of amines derived from 5-nitroindazole [1-(aminoalkyl)indazolinones, 3-(aminoalkoxy) indazoles and 3-(alkylamino)indazoles]	IC_{50} less than 50 µM	In vitro	Solution	ESCARIO GARCÍA-TREVIJANO JOSÉ ANTONIO	WO2019077174A1	[127]

Table 2. *Cont.*

Active	Dose	Testing Method	Pharmaceutical Form	Inventor (Patent Applicants)	Identification	Reference
Natural Products						
Coix seed, jade grass, gentian, gorgon, purslane, hundreds skin, gardenia, anemarrhena, white fresh leather, phellodendron, cnidium, guan zhong, tactylodes, chrysanthemum, lotus seed, plant grass, licorice, peony skin, rehmannia, bai wei, sophora	Multidose	Human	Complex mixture	LI SHAOLUN	CN106668673A	[128]
Earthworms, cnidium, *Sophora flavescens* aiton, white fresh skin, berberine, 100 parts, phellodendron, chuanjiao, chuanpi	Multidose	NM	Herbal mix	THE INVENTOR HAS WAIVED THE RIGHT TO BE MENTIONED	CN105343717A	[129]
Snake bed, Wuyu, Honey, realgar	Snake bed 35–55%, wuyu 10–15%, honey 31.85–49.7%, realgar 0.15–0.3%	NM	Nanopill	CHANG HAOLIANG; FENG TIANBAO	CN102274327A	[130]
Water spinach, ampelopsin grossedentata, *Haloragis micrantha* (Thunb.) R.Br. ex Sieb. and Zucc, malabar spinach, *Silene gallica* L., root of pilular adina, *Ajuga taiwanensis* nakai ex murata, herb of prostrate euphorbia, willow root, common nandina leaf, wing nut leaf, David's buddleia, *Chenopodium album* L., sensitive joint vetch wood, *Hedyotis diffusa* Willd, *Adiantum davidii* Franch. and *Pteridium revolutum* (Blume) Nakai	Multidose	In vivo	Herbal mix	XUE JIANFANG; ZONG XIUHONG; FENG ZUOJI; YANG HAIXIA; CHU JINGPING	CN104740113A	[131]
Nanotechnology						
Oil-in-water phellodendron oil nanoemulsion	Phellodendron oil 5.8%	In vivo	Nanoemulsion	WUQING OUYANG	CN102397379A	[132]

A total of twelve patents were found; seven presented the application of synthetic drugs, some of which are already recommended in trichomoniasis treatment. Patent applications are usually made by the university or the inventor, while companies participate to a lesser degree, with greater attention aimed at compounds of synthetic origin (Figure 3). In those cases, the authors propose a new pharmaceutical form based on commercial drugs in order to propose a more targeted delivery system for the vaginal mucosa. Special attention has been given to the area of biomolecules and nanotechnology for the treatment of trichomoniasis, even though these documents represent a lower total value. This is a technology with more recent applications in the pharmaceutical field when compared to drug synthesis.

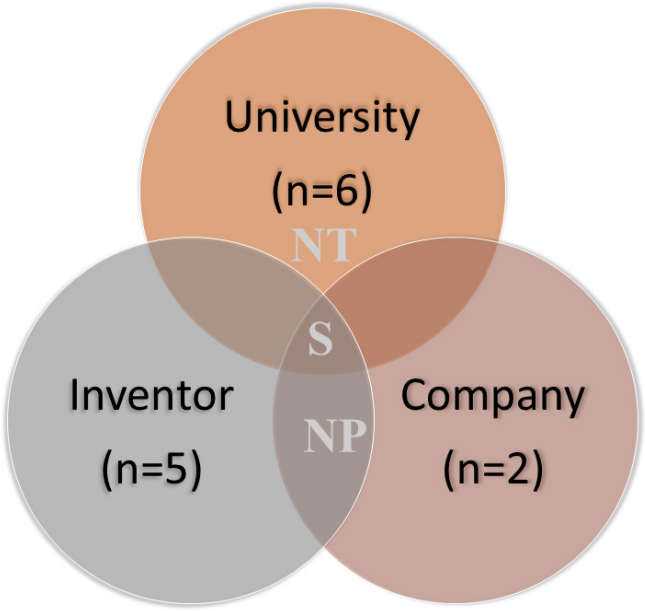

Figure 3. Venn diagram demonstrates the overlapping of patent applicant origin. NT: nanotechnology, NP: natural products, and S: synthetic compounds.

3.2.1. Patents: Synthetic Compounds

Rational planning is one of the strategies used to synthesize new compounds with pharmacological action against *T. vaginalis*. A key enzyme in the pathogen metabolism is triosephosphate isomerase, which may be the target of new potential drugs under investigation. In this sense, recent research (WO2018065809A1; WO2018065807A1; WO2018065808A1) has focused on inhibiting the action of this enzyme [123–125]. In the three documents found, a rational search was made of compounds that interact with the extracellular *T. vaginalis* triosephosphate isomerase. New anti-trichomonad molecules were found that were capable of altering the growth and viability (in vitro tests) of cultures of *T. vaginalis* in 51 patients.

In the first invention (WO2018065809A1), two different compounds were synthesized via computer programs (virtual docking) [124]. The compounds 3′-{[4-morpholinyl)phenyl] methylene}bis(4-hydroxy-2*H*-chromen-2-one) (A4) and 5,5′-[(4-nitrophenyl)methylene]bis(6-hydroxy-2-mercapto-3-methyl-4(3*H*)-pyrimidinone) (D4) and their derivatives have shown an inhibitory action on the central metabolism of *T. vaginalis*, especially when used concomitantly (IC_{50} 48 µM). The compounds showed action after 3 h of exposure to the isolates, which is better than MTZ, which demonstrates action within 4–6 h. In another document, the same authors (WO2018065807A1) synthesized the molecule 3,3′-{[4-(4-morpholinyl) phenyl] methylene} bis (4-hydroxy-2*H*-chromen-2-one) (A4), which showed tricomonacidal action at up to 24 h with an IC_{50} of 47 µM. The authors suggested that this compound could be used for topical application as a cream or gel. However, all analyses were performed with the compound solubilized in DMSO as a solvent [123]. Finally, the same research group, in another patent (WO2018065808A1), proposed the application of the compound 5,5′-[(4-nitrophenyl) methylene] bis (6-hydroxy-2-mercapto-3-methyl-4 (3*H*)-pyrimidinone (D4) in *T. vaginalis* (IC_{50} 153 µM at 24 h). In all cases, it was determined that the compounds did not present mutagenic, cytotoxic, or carcinogenic effects at the concentrations tested [125].

Another strategy is to combine the effects of new molecules with drugs already used in treating trichomoniasis to increase the therapeutic effect. In this sense, the invention US2010055201A1 reported an increased anti-*T. vaginalis* effect when the compound diindolylmethane (DIM) and DIM-related indoles were combined with other antiprotozoa [126]. The authors suggest that this substance may have a combined or synergistic action with one or more antiprotozoal agents, such as atovaquone, amodiaquine, amphotericin B, butoconazole and clindamycin among others. The selective elimination of infected

cells led to a consequent decrease in parasite-caused lesions. This effect can be seen using DIM, in a concentration range between 100 and 200 mg applied orally once or twice a day for 1–2 weeks, together with standard doses of MTZ. However, the dose and frequency of administration will depend on the type of treatment used [126].

In the invention WO2019077174A1, the authors proposed the combination of three different molecules (derived from 5-nitroindazole amine families) for manufacturing drugs to treat parasitic infections (Chagas Disease, African sleeping sickness, leishmaniasis), including trichomoniasis [127]. These compounds differ because they have specific substitutions in the molecule, such as alkylamino or dialkylamino groups, and present advantages over the currently used drugs because of their chemical structure. They were designed to be water-soluble, because they have primary, secondary, or tertiary groups capable of forming salts with appropriate inorganic or organic acids. The authors described the synthesis routes of the compounds from substances known as 2-benzyl-5-nitroindazolinones, among others, and the in vitro anti-*T. vaginalis* activity of indazole derivatives was demonstrated. First, the compounds were evaluated against MTZ-sensitive trophozoites, and simultaneously against Vero cells, to detect nonspecific cytotoxicity. The compounds showed IC_{50} values less than 50 μM, and represent a promising, safe, and potent pharmacological approach [127].

The invention CN106667983A demonstrated the action of salt 1,6-bis(N1-p-chlorophenyl-N5-biguanidino) hexane on *T. vaginalis*. The compound was formulated as an aqueous acetate solution at concentrations between 1%, 0.1% and 0.01% (*m*/*v*), and as a purified aqueous gluconate solution at concentrations of 1%, 0.1% and 0.05% (*m*/*v*). In both strategies, patients with vaginitis caused by *T. vaginalis* were treated with the formulations once daily for seven days, and the results showed that the patients' symptoms diminished or disappeared [121].

Thies technology can also be used as a way to improve the effectiveness of existing drugs. For example, the invention US20200289470A1 proposed the use of secnidazole in the form of microgranules (sizes between 400 and 841 μm). The microgranules were formulated using sugar spheres, providone, polyethylene glycol 4000, ethyl acrylate methylacrylate, Eudragit NE 30D, and other components. The formulation was tested in women with *T. vaginalis*, pregnant or not, with four recurrent episodes in 12 months, and in women with a confirmed diagnosis of bacterial vaginosis. The pharmacokinetic profile of secnidazole was studied after the administration of 2 g as a single dose. The results showed the better effects of this approach compared to drugs already approved by the FDA [133].

The invention ES2653674B2 presented three novel molecules with antiprotozoal properties, including anti-trichomoniasis produced from 5-nitroindazole. They are the amine derivatives (1-aminoalquil)indazolinonas, 3-(aminoalcoxi)indazoles and 3-(alquilamino)indazoles. The compounds were designed to have higher water solubility and better pharmacokinetics profiles. These results were achieved by introducing primary, secondary, or tertiary amino groups to the molecules responsible for producing water-soluble properties [122].

3.2.2. Patents: Natural Products

Biomolecules have been attracting much interest from researchers, especially for the use of treating diseases such as trichomoniasis, since this STI has been associated with high rates of resistance to synthetic drugs. In our research, three patents proposed the application of biomolecules as anti-*T. vaginalis* treatments (CN106668673A; CN105343717A; CN104740113A) [128,129,131]. These biomolecules are of natural origin and were obtained from plants with great biodiversity. Interestingly, all the proposals consist of combinations of active extracts from medicinal plants.

The invention CN106668673A consists of a complex mixture of different assets with great biodiversity. All assets can be used in different concentration ranges. For example, gentleman (10–20 g), coix seed (10–15 g), juncus (5–15 g), gentian (5–15 g), gorgon (10–15 g), purslane (10–20 g), child (15–20 g), gardenia (5–15 g), anemarrhena (5–15 g), white

fresh skin (10–15 g), cork (15–20 g), cnidium (10–20 g), guanzhong (10–15 g), atractylodes (5–15 g), chrysanthemum (5–10 g), lotus seeds (10–15 g), plantain grass (10–15 g), licorice (10–20 g), moutan (5–15 g), ground yellow (10–15 g), white wei (10–20 g) and sophora (10–14 g). A total of 23 patients infected with *T. vaginalis* were treated with a mixture of compounds, and the effective rate was 76.19%; four cases were improved, and the effective rate was 95.23% [128].

The use of a mixture of 12 medicinal herbs used in Chinese medicine has been proposed to treat vaginitis caused by *T. vaginalis* (CN105343717A). The mixture consists of 30 g of earthworms, 30 g of cnidium, 15 g of *Sophora flavescens*, 15 g of white fresh skin, 10 g of berberine, 20 g of 100 parts, 20 g of phellodendron, 15 g of chuanjiao, 15 g of chuanpi G, and 20 g of withered [129].

The invention CN104740113A involved extracting vegetable oils containing different chemical compounds to produce an ointment. The raw materials included in the invention are: leek, rattan tea, two small types of grasses, fly grass, water group, blood grass, willow root, southern bamboo leaves and willow leaves, among other substances [131]. Another invention that consists of a similar proposal, that is, based on Chinese medicine (CN102274327A), used the following raw materials: cnidium fruit, fructus evodiae, honey, and realgar. In addition, the authors described the compost preparation process, which involves mixing all raw materials. This process is based on three steps: weighing the raw materials, mixing, incorporating them with honey, and producing the tablets [130].

3.2.3. Patents: Nanotechnology

Recently, nanotechnology has been proposed as an alternative to carrying drugs with antiprotozoal action, in order to increase the drug's residence time within the disease site and increase its effect.

The proposal of a nanoemulsion containing a combination of different oils was recently made (CN102397379A). The system consisted of particles of sizes between 1 and 100 nm containing phellodendron, which provides a high penetration rate, good bioavailability, and high active stability for the treatment of *T. vaginalis* infection. The emulsion composition involves mixing different compounds such as phellodendron oil 5.8%, cinnamaldehyde 0.3%, camphor oil 0.4%, terbinafine 0.7%, olive oil 0.7%, ethanol 0.6%, castor oil 27.5%, distilled water 64.0% and polyoxyethylene ether hydrogenate. Although the authors indicated the formulation for treating trichomoniasis, they only performed studies in animals (sheep) with skin diseases/lesions [132].

The development of new technologies represents a fundamental element of growth, differentiation, and problem-solving strategies, especially for a disease such as trichomoniasis. In this way, when there is the creation of something new, such as the development of a manufacturing process or the improvement of existing techniques and products, protection is provided through patents [134,135]. Therefore, the patent is considered a temporary privilege granted to inventors, characterized by the exclusive right to exploit the technology; however, the invention becomes public [136].

Patent prospecting represents a complete source of research, as it points out the areas and services that society needs. In addition, the analysis of documents such as patents demonstrates the dissemination of practical and economic knowledge, the encouragement of scientific research, and formation of new areas [137]. In this context, prospecting studies are of great relevance as they provide status and growth trends in an area of knowledge or product of interest. The survey of information on new therapeutic approaches also enables scientific advancement, expands anticipation capacity, and helps track the applicability of new technologies [135,136]. When treating trichomoniasis, the search for data in patents helps to map the scientific and technological evolution of formulations that can significantly influence the future field.

Finally, in the search, we observed a predominance of patents related to synthetic drugs. However, other methodologies have emerged to accelerate the treatment process, offering new advantages in final pharmaceutical formulations.

3.3. Clinical Trials

The search for potential new alternatives for treating trichomoniasis via clinical trials revealed one observational and six interventional studies from the last ten years (Table 3). Most of these studies involved treatments with the drugs already used in therapy, MTZ and tinidazole, or secnidazole, all from the same 5-nitroimidazole class. In this sense, it is possible to observe that no studies were found involving the evaluation of new therapeutic targets and/or the use of nanotechnology systems. One study (NCT03935217), developed between April 2019 and March 2020 with 147 women enrolled at ten sites in the USA, aimed to evaluate the effectiveness and safety of a single oral dose of secnidazole (Solosec®) for the treatment of trichomoniasis. This double-blind study was multi-center, prospective, randomized and placebo-controlled [138]. The results revealed that a single oral 2 g dose of secnidazole produced higher cure rates than placebo, including in those with HIV and/or bacterial vaginosis [60]. Another phase III randomized clinical trial (NCT01832480) determined the influence of patient treatment and host factors on repeating *T. vaginalis* infections among HIV-negative women. HIV-negative women (623 randomized) who tested positive for *T. vaginalis* at their routine gynecological exam were distributed into two groups: MTZ 2 g single dose (270 completed treatments) or MTZ 500 mg twice daily for 7 days (270 completed treatments). The last regimen showed better results [139].

Table 3. Clinical trials testing potential new alternatives to treat trichomoniasis.

Active/Formulation	Dose	Phase	Pharmaceutical Form	Identification	Ref
Clinsupv	Clindamycin 100 mg and clotrimazole 200 mg (both administered per vaginally for 3 consecutive days)	4	Soft gelatin capsule *versus* extended release tablet	NCT01697826	[140]
Drug: iptp-sulphadoxine-pyrimethamine plus metronidazole Drug: iptp-dihydroartemisinin-piperaquine plus metronidazole drug: iptp-sulphadoxine-pyrimethamine	SP = 3 tablets each containing 500 mg sulphadoxine and 25 mg pyrimethamine (Day 0) MTZ = 4 tablets each containing 500 mg as directly observed therapy (Day 0) DP = 3 tablets of 40 mg of dihydroartemisinin and 320 mg of piperaquine (Days 0, 1, 2)	3	Tablets	NCT04189744	[141]
Gynomax® XL	Lidocaine 100 mg, thioconazole 200 mg, tinidazole 300 mg	4	Vaginal ovule	NCT03839875	[142]
Metronidazole	500 mg twice daily for 7 days or 2 g single dose	3	Oral	NCT01832480	[139]
Neo-Penotran Forte	Metronidazole 750 mg and miconazole nitrate 200 mg	2	Vaginal suppository	NCT01361048	[143]
Neo-Penotran® Forte	Metronidazole 750 mg and miconazole nitrate 200 mg	Observational	Vaginal suppository	NCT01335373	[144]
Solosec (Secnidazole) or placebo	2 g	3	Oral granules	NCT03935217	[138]

Clinical trials on new drug route administration for *T. vaginalis* infection were also carried out. The combination of a vaginal product with a higher dose of MTZ with miconazole (Neo-Penotran Forte) (NCT01361048) was evaluated in order to test its effectiveness in treating trichomoniasis. Forty participants were enrolled in three groups: (i) MTZ 2 g oral single dose; (ii) Neo-Penotran Forte intravaginally twice a day for 7 days; (iii) Neo-Penotran Forte intravaginally once a day for 7 days [143].

The efficacy and safety of a Gynomax® XL ovule containing tioconazole, tinidazole and lidocaine was tested to treat trichomonal vaginitis, bacterial vaginosis, candidal vulvovaginitis and mixed vaginal infections in an open-label, single-arm, multicentral study (NCT03839875). One hundred and sixteen participants were enrolled, and the study had no results (either posted on ClinicalTrials.gov or published by the study completion date on 9 August 2019) [142]. The observational, cohort, prospective study enrolled 13,024 participants who had microbiological tests positive for vaginal candidiasis, bacterial vaginosis, or trichomoniasis, to test the efficacy and safety of MTZ/miconazole (Neo-Penotran Forte) (NCT01335373). Although the study's completion date was April 2015, the results were not posted on ClinicalTrials.gov [144]. The comparison of two topical formulations containing 200 mg clotrimazole and clindamycin phosphate equivalent to 100 mg clindamycin for 3 days, in 73 patients with vaginal infections, was conducted to test efficacy and tolerability (NCT01697826). That study was a randomized, comparative, prospective, open-label, single-center study, seeking to compare the 3-day treatment course of an intravaginal soft gelatin capsule containing clindamycin and clotrimazole with intravaginal extended-release tablets of clindamycin and clotrimazole in patients with vaginal discharge and a clinical diagnosis of bacterial, trichomonal, candidal or mixed vaginitis. As for the other two clinical trials, the results were not posted on ClinicalTrials.gov, even by the study's completion date on September, 2011 [140].

One study, on ClinicalTrials.gov, that was still recruiting participants at the time of this review is the ASPIRE Trial, aiming at safe pregnancies by reducing malaria and infections in the reproductive tract. This is an individually randomized, three-arm, partially placebo-controlled trial (NCT04189744) intending to compare the efficacy, safety, and tolerability of using intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP), versus MTZ or IPTp-dihydroartemisinin-piperaquine (DP) with MTZ, to reduce adverse birth outcomes attributable to malaria and STIs. The estimated enrollment is 5436 participants from the Nchelenge District of Zambia, with the study completion date set as 7 November 2022 [141].

4. Conclusions

Trichomoniasis is the most common STI of non-viral origin in the world. The global estimate of infection in 2016 was an incidence of 156 million new cases. However, these data are underestimated, because trichomoniasis is not notifiable, receiving little attention from public health programs seeking to control STIs, and therefore, it is considered a neglected parasitic infection by the CDC-USA. FDA-recommended treatments include MTZ and TNZ; recently, secnidazole joined this list [145,146]. So far, there are no options for the oral treatment of trichomoniasis other than 5-nitroimidazoles, as mentioned above.

Although trichomoniasis is considered a curable STI, therapeutic failure rates are high. These include the resistance of *T. vaginalis* isolates to the recommended drug, MTZ, estimated at 2.5 to 9.6%. This scenario generates the worrying numbers of 160,000 people in the USA and approximately 10 million worldwide in need of alternative treatment. In this context, the search for new targets and molecules with therapeutic potential to control trichomoniasis is extremely relevant on the world stage. Although MTZ and TNZ represent the conventional treatments for this disease, they are associated with adverse effects and consequent non-adherence to the treatment.

Overall, the data compiled in this review highlight several clinical trials continuing to test new routes of administration for current FDA-approved drugs. The control of the most common non-viral STI depends on new treatment options with novel therapeutic targets to minimize current problems, such as well-established resistance and therapeutic failures. Most of the articles and patents found in the present review demonstrated the effectiveness of new synthetic compounds and NP in experimental models in vitro, but the lack of an in vivo model for trichomoniasis still impairs the progress in this area. The encouraging results generally demonstrate a better effect of these new molecules compared to conventional treatments. However, these newly proposed approaches need, in addition

to pharmaceutical development and efficacy assessments in animal models and patients, to ensure that the quality requirements for their use as medicines are met. It is essential to overcome these issues to cross the "Death Valley" of drug discovery, and to proceed in the translational science of the trichomoniasis drug development field. In the last decade, the only successful case of a translational study on trichomoniasis treatment was on secnidazole, which was approved in 2017 by the FDA to treat bacterial vaginosis, and was recently (June, 2021) approved for the treatment of *T. vaginalis* infection (Figure 4) [145,146]. In this sense, pharmaceutical nanotechnology could shed light on new efficient formulations, offering an improved bioavailability of drugs and reductions in adverse effects through topical administration. Furthermore, this update shows that works on NP have focused on searching for new therapeutic options to treat *T. vaginalis* infection, the majority of which come from plants. However, interesting NP of marine origin could also be considered as potential new drugs.

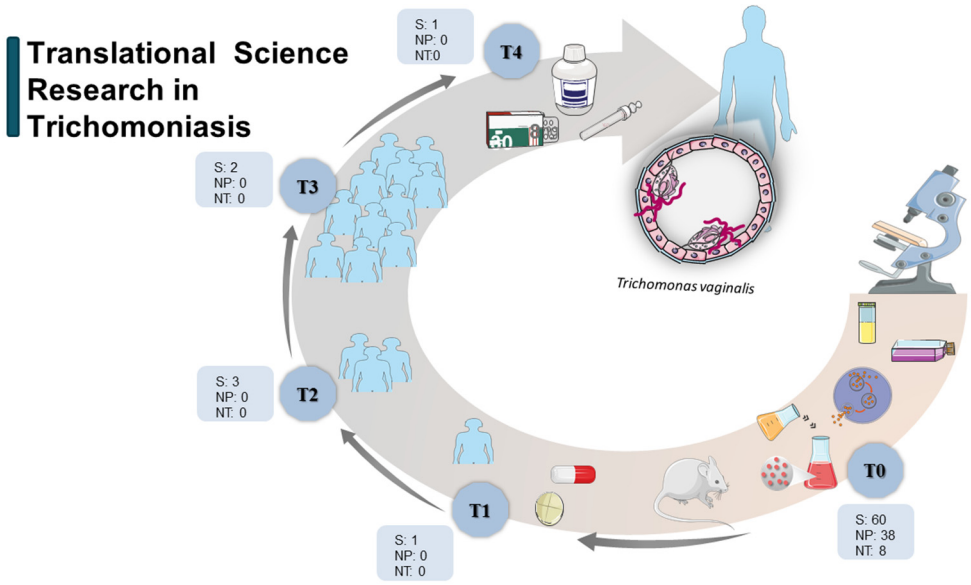

Figure 4. Translational research on drug development against the parasite *Trichomonas vaginalis*, from laboratory bench to bedside. T0: basic research and drug discovery. T1–T3: clinical research through clinical trials phases I and II (T1), III (T2), and IV (T3). T4: clinical implementation with new drugs and health products. The numbers are related to reviewed studies with synthetic compounds (S), natural products (NP), and nanotechnology (NT) approaches.

Finally, research groups dedicated to developing new therapeutic alternatives for this neglected STI are producing relevant results. Efforts should be encouraged in terms of boosting basic research, developing pharmaceutical formulations, and performing clinical studies on the translational process from the bench to the patient, thus improving health policies.

Author Contributions: T.T., A.L.S.d.S. and G.V.R. conceived the study; T.T., G.V.R. and L.A.F. designed the article; G.V.R. and L.A.F. searched the data; G.V.R. and G.B.G. constructed the figures and tables; T.T., G.V.R., L.A.F. and G.B.G. drafted the article; A.L.S.d.S. revised it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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