Caatinga plants: Natural and semi-synthetic compounds potentially active against *Trichomonas vaginalis*

Patrícia de Brum Vieira, Nícolas Luiz Feijó Silva, Gloria Narjara Santos da Silva, Denise Brentan Silva, Norberto Peporine Lopes, Simone Cristina Baggio Gnoatto, Márcia Vanusa da Silva, Alexandre José Macedo, Jaume Bastida, Tiana Tasca

PII: S0960-894X(16)30281-5
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.03.061
Reference: BMCL 23704

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 12 November 2015
Revised Date: 14 March 2016
Accepted Date: 15 March 2016

Please cite this article as: Vieira, P.d.B., Silva, N.L.F., Silva, G.N.S., Silva, D.B., Lopes, N.P., Gnoatto, S.C.B., Silva, M.V.d., Macedo, A.J., Bastida, J., Tasca, T., Caatinga plants: Natural and semi-synthetic compounds potentially active against *Trichomonas vaginalis*, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.03.061

Caatinga plants: natural and semi-synthetic compounds potentially active against *Trichomonas vaginalis*

Patrícia de Brum Vieira[a], Nícolas Luiz Feijó Silva[a], Gloria Narjara Santos da Silva[b], Denise Brentan Silva[c,d], Norberto Peporine Lopes[d], Simone Cristina Baggio Gnoatto[b], Márcia Vanusa da Silva[e], Alexandre José Macedo[f,g], Jaume Bastida[h], Tiana Tasca[a,g]*

[a]Laboratório de Pesquisa em Parasitologia, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90610-000, Brasil; Emails: patriciasbrum@yahoo.com.br; nicolaslfsilva@gmail.com; tiana.tasca@ufrgs.br.

[b]Laboratório de Fitoquímica e Síntese Orgânica, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90610-000, Brasil; Emails: narjaragS@gmail.com; simone.gnoatto@ufrgs.br.

[c]Laboratório de Produtos Naturais e Espectrometria de Massas (LAPNEM), Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS, 79090-900, Brasil; Email: denise.brentan@ufms.com.

[d]Núcleo de Pesquisas em Produtos Naturais e Sintéticos, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903, Brasil; Emails: npelopes@fcfrp.usp.br .

[e]Centro de Ciências Biológicas e Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife, PE, 50670-901, Brasil; Email: Márcia.vanusa@ufpe.br.

[f]Faculdade de Farmácia e Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90610-000, Brasil; Email: Alexandre.jose.macedo@gmai.com.

[g]Instituto Nacional do Semi-Árido (INSA), Núcleo de Biprospecção da Caatinga (NBioCaat), Campina Grande, Pernambuco, Brasil.

[h]Department of Natural Products, Plant Biology and Soil Science, Faculty of Pharmacy, Barcelona, Catalonia, 08028, Spain; Email: jaumebastida@ub.edu.

*Author to whom correspondence should be addressed, email tiana.tasca@ufrgs.br; Phone: +55 51 3308 5325; Fax: +55 51 3308 5437.

Abstract

Trichomonas vaginalis causes trichomoniasis; the most common but overlooked non-viral sexually transmitted disease worldwide. The treatment is based at 5'-nitroimidazoles, however, failure are related to resistance of *T. vaginalis* to chemotherapy. Caatinga is a uniquely Brazilian region representing a biome with type desert vegetation and plants present diverse biological activity, however, with few studies. The aim of this study was to investigate the activity against *T. vaginalis* of different plants from Caatinga and identify the compounds responsible by the activity. A bioguided fractionation of *M. rufula* was performed and four major compounds were identified*:* caproate of α-amyrin (**1b**), acetate of β-amyrin (**2a**), caproate of β-amyrin (**2b**), and acetate of lupeol (**3a**). In addition, six derivatives of α-amyrin (**1**), β-amyrin (**2**) and lupeol (**3**) were synthesized and tested against the parasite. Ursolic acid (**5**) reduced about 98% of parasite viability after 2h of incubation and drastic ultrastructural alterations were observed by scanning electron microscopy. Moreover, **5** presented high cytotoxicity to HMVII and HeLa cell line and low cytotoxicity against Vero line at 50 μM (MIC against the parasite). Metronidazole effect against *T. vaginalis* resistant isolate was improved when in association with **5**.

Keywords: *Trichomonas vaginalis*; Caatinga plants; triterpenes; ursolic acid; cytotoxicity.

Abbreviations: ^{1}H or ^{13}C NMR, proton or carbon nuclear resonance magnetic; $CDCl_3$, deuterated chloroform; TMS, tetramethylsilane; EI, electron ionization; MS, mass spectroscopy; GC, gas chromatography; RI, retention index; IR, infrared spctra; IPA, Instituto Agronômico de Pernambuco; VLC, vaccum liquid chromatography; EtOAc, ethyl acetate; MeOH, methanol; TLC, thin liquid chromatography; DMAP, 4-dimethylaminopyridine; **(1)**, α-amyrin; **(2)**, β-amyrin; **(3)**, lupeol; **(1a)**, acetate of α-amyrin; **(1b)**, caproate of α-amyrin; **(2a)**, acetate of β-amyrin; **(2b)**, caproate of β-amyrin; **(3a)**, acetate of lupeol; **(3b)**, caproate of lupeol; DMSO, dimethyl sulfoxide; TYM, trypticase-yeast maltose medium; SEM, scanning electron microscopy; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Trichomonas vaginalis is a flagellated protozoan that causes trichomoniasis, the number one non-viral sexually transmitted disease worldwide.[1] The parasite colonizes the human tract and causes vaginitis in women and urethritis in men. Serious consequences are related to trichomoniasis, including cervical and prostate cancers increased risks,[2] adverse pregnancy outcomes and an increased susceptibility to human immunodeficiency virus acquisition.[3] Metronidazole, an antibiotic of the nitroimidazole class, is the first-line treatment for trichomoniasis. Nevertheless, *T. vaginalis* resistance to metronidazole is rising[4] and requires new strategies to treat this neglected infection. Natural products play an important role in the search for new active drugs.

Caatinga is a semi-arid region of Brazil Northeastern with a great diversity of plants and animals, however it is yet poorly studied and its potential is underestimated. The plants *Croton nummularius* Baill*., Senna lechriosperma* H.S.Irwin & Barneby, and *Manilkara rufula* (Miq.) H.J. Lam, Blumea are native and endemic of Northeast of Brazil. Several biological activities have been attributed to plants of *Croton, Senna* and *Manilkara* genera*,* such as anti-inflammatory and analgesic,[5] anti-microbial, anti-

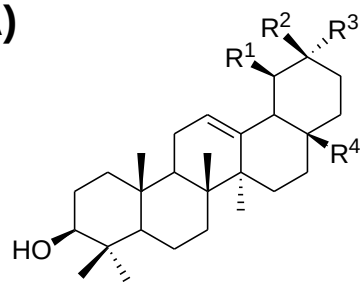
parasitic, anti-HIV and antitumoral.[6] Moreover, anti-*T. vaginalis* activity of *M. dissecta* crude extract was reported.[7] The necessity of studies in this area is requiring, because there is a scarcity of studies about Caatinga plants.

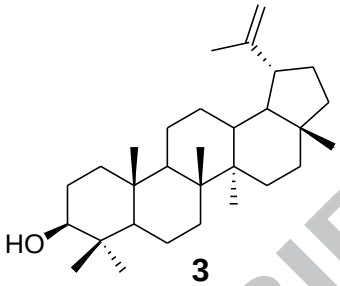
The miscellaneous of activities related to *Manilkara* sp. may be attributed to the presence of a range of secondary metabolic in these species, such as saponins, flavonoids, tannins, and triterpenes.[8] These compounds present a variety of biological activities, such as anti-inflammatory, antiviral, antibacterial, antifungal, and antitumor.[9] Moreover, triterpenes showed potential activity against protozoa, such as *Leishmania, Trypanosoma, Plasmodium* and *Trichomonas vaginalis.*[10,11] Triterpenes are important candidates for designing lead compounds for new active agents development, as showed by Guimarães *et al.*[12] Moreover, structural modifications approaches help to improve biological activities against etiological agents and, nowadays, have showed an increasing interest.

In order to investigate the potential against *T. vaginalis,* leaves and branches of *C. nummularius, M. rufula* and *S. lechriosperma* were collected at Parque Nacional do Catimbau (PARNA do Catimbau), Pernambuco, Brazil (8°37'S 37°08'W) in February 2012 with authorization of SISBIO 16.806 Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). Voucher specimens have been deposited in the Herbarium of the Instituto Agronômico de Pernambuco (IPA) as *C. nummularius* (IPA 86868), *M. rufula* (IPA 84889) and *S. lechriosperma* (IPA 84959). The dried leaves and branches of the three plants were powered and extracted with ethanol:water. Polar components were removed by liquid-liquid extraction with acetate and *n*-butanol. Non-polar fraction was subjected to a sequential vacuum liquid chromatography (VLC).[13]

In addition, six derivatives of α-amyrin **(1)**, β-amyrin **(2)**, and lupeol **(3)** were synthesized[14] (Figure 1A) and the compounds identification was performed by IR, ^{1}H and ^{13}C NMR, and HR-ESI-MS[15-20]. The data were compared with previous study.[21]

(A)

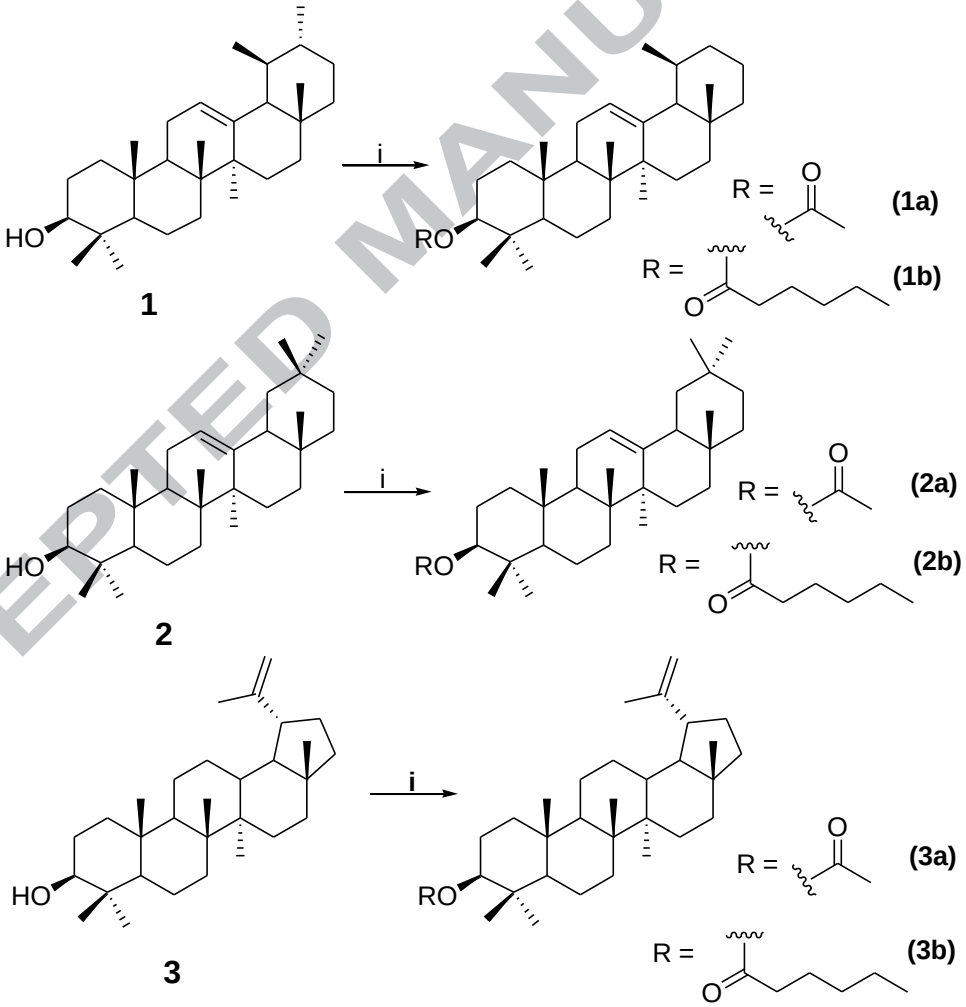




3

1: R^1 = Me, R^2 = H, R^3 = Me, R^4 = Me
2: R^1 = H, R^2 = Me, R^3 = Me, R^4 = Me
4: R^1 = H, R^2 = Me, R^3 = Me, R^4 = COOH
5: R^1 = Me, R^2 = H, R^3 = Me, R^4 = COOH

(B)



Reagents and conditions: (i) dichloromethane, commercial anhydride (acetic or caproic anhydride), DMAP, reaction time of 3 h

Figure 1.

The parasites used in this study were *Trichomonas vaginalis* 30236 metronidazole-sensitive isolate from ATCC and TV-LACM2R metronidazole-resistant isolate (fresh clinical isolates from female patients from Laboratório de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, UFRGS, Brazil; project with ethical approval by UFRGS Ethical Committee, number 18923). Trophozoites were cultured as previously described by Diamond.[22] Anti-*T. vaginalis* assays were performed.[23] Moreover, the minimum inhibitory concentration (MIC) of ursolic acid (**5**) was determined by eightfold dilution, ranging of 100 – 0.78 µM. After 24 h of incubation, the viability was evaluated by counting in hemocytometer with exclusion dye trypan blue 0.2% and comparing with parasites untreated. After MIC determination, parasites were inoculated to fresh TYM medium and MIC was confirmed. The effect of **5** on parasite growth and viability was performed.[24] In addition, the viability of the parasites was assessed using fluorescein diacetate (FDA) (Sigma, USA) and propidium iodide (PI) (Sigma, USA). Parasites were treated or not with **5** at 50 µM for 0, 2, 4, 6, and 12 h and stained with 10 µg/mL of FDA and 10 µg/mL of PI for 20 min for fluorescence analysis.

In order to verify parasite ultrastructure changes induced by **5**, scanning electron microscopy (SEM) was performed as described in previous study with *T. vaginalis.*[25] Also, the cytotoxicity of UA to HMVII (vaginal epithelial melanoma cell), HeLa (cervical cancer cell), and VERO (kidney epithelial cells) lines was performed.[26] The association of **5** and metronidazole was performed against TV-LACM2R isolate at 6.25 or 12.5 µM of **5** in association or not with 15 and 73 µM metronidazole. All the experiments were performed in triplicate and with at least three independent cultures (n = 3). Student's *t* test was chosen for comparisons between two groups. The results are expressed as the mean ± SEM of at least three individual experiments. $P < 0.05$ was considered a statistically significant difference. Analyses were performed using Statistical Package for the Social Sciences (SPSS) software v.14.

Ethanolic extracts of leaves and branches of *C. nummularius* and *S. lechriosperma* showed a slight reduction (about 40%) of *T. vaginalis* viability at 1.0 mg/mL (Figure 2A). Studies demonstrated that different species of *Croton* and *Senna* present a range of biological activities,[5] however, we did not find reports about anti-*T. vaginalis* activity of *Senna* sp. nor *Croton* sp.

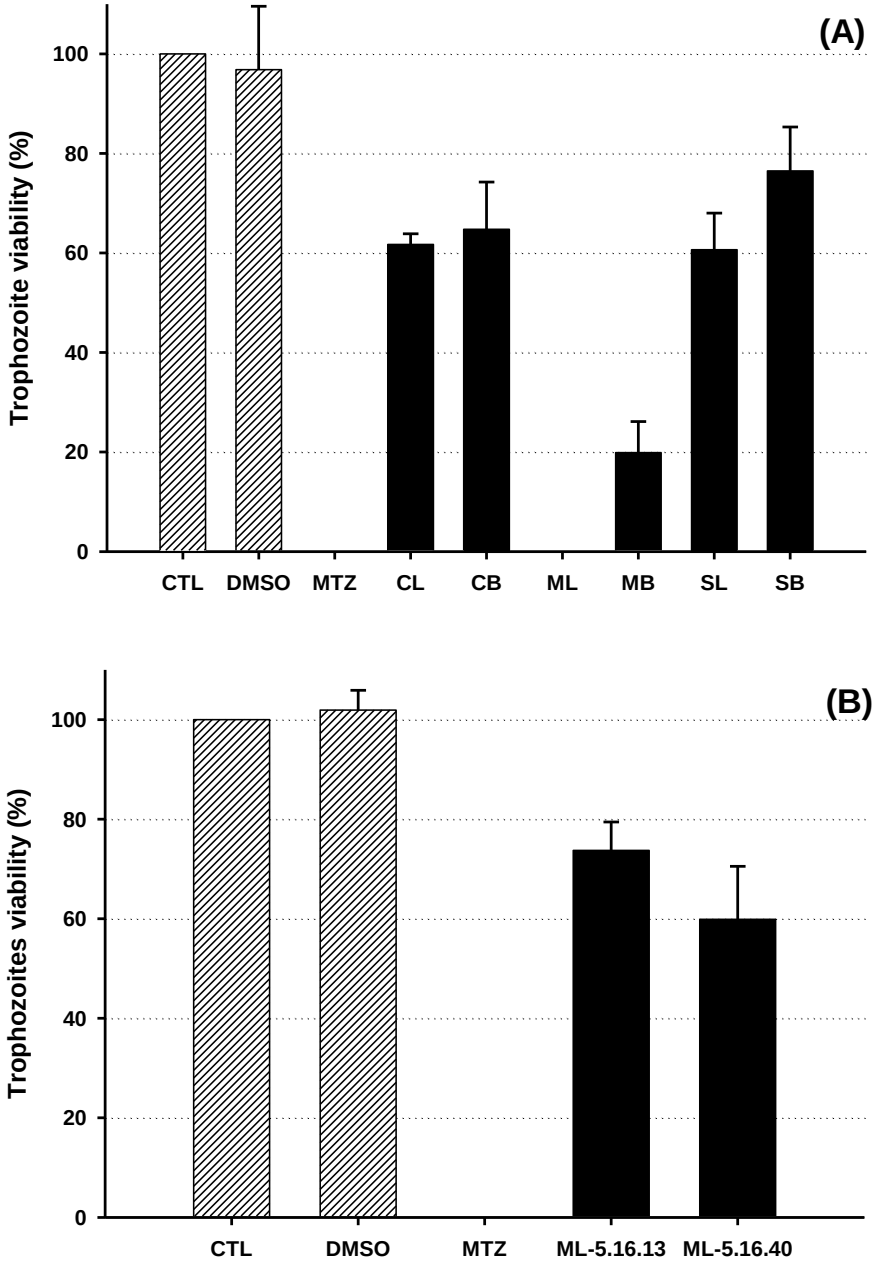


Figure 2.

In the case of *M. rufula* extracts (leaves and branches), at 1.0 mg/mL, the parasite viability was reduced up to 100% and 80% (Figure 2A), respectively, showing a promising activity of *M. rufula* extracts against *T. vaginalis.* These results agreed with the literature, which was demonstrated anti-*T. vaginalis* activity of *M. dissecata.*[7] Taking into account the potential of *M. rufula* extract against the parasite, the crude extract of this plant was bioguided fractionated. After, repeated column chromatography on silica gel was performed, two major triterpene-enriched fractions were obtained (ML-5.16.13 and ML-5.16.40). These fractions promoted a low reduction of parasite viability, 26.3% and 40.1%, respectively (Figure 2B). Regarding the scarcity of chemical studies from *M. rufula*, GC-MS and NMR analysis were performed to identify the mainly compounds in the triterpene-enriched fractions. Taking into account that these compounds are largely found in nature and their structures are already known, the *M. rufula* compounds identification was based in literature comparison: **1b**, **2a**, **2b**, and **3a** (Figure 1B).[27] Despite these compounds are known, it is the first time that these compounds were isolated and identified in *M. rufula*, highlighting the importance of such study of plants from Caatinga region. Many plants from Caatinga show a great potential, however, the phytochemical and biological properties from the most of these plants have never been studied before.

In attempting to improve *M. rufula* fractions biological activity and to characterize the activity of isolated compounds, six derivatives based on **1**, **2**, and **3** (Figure 2A) structures were synthesized: **1a**, **1b**, **2a**, **2b**, **3a,** and **3b** (Figure 2B). This strategy is considered a hot research topic in medicinal chemistry over ten decades and demonstrated the potential of natural compounds derivatives as leading compounds.[11,28] The anti-*T. vaginalis* activity of the six synthesized derivatives, the standards **1**, **2**, and **3** and derivatives acids oleanolic **(4)** and ursolic acid **(5)** was performed. As demonstrated at Table 1, semi-synthetic compounds demonstrated a low reduction of the parasite viability. The compound **3b** presented the lowest reduction of

T. vaginalis viability, 28.6% at 100 µM, in comparison with negative control. The other compounds reduced about 40% of trophozoite viability. Although data from literature demonstrate that derivatives from different skeleton have different biological activity,[29] herein, we demonstrated that derivatives obtained from three different skeleton showed similar anti-*T. vaginalis* activities. The compounds **1**, **2**, **3** and **4** demonstrated a weak reduction of *T. vaginalis* viability, representing a reduction of less than 25% the parasite viability at 100 µM (Table 1).

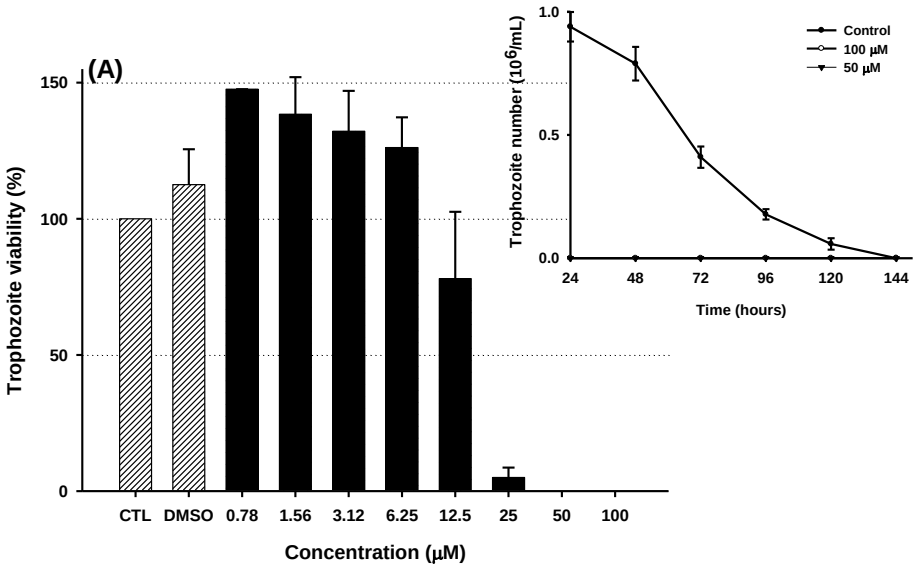
Table 1. Anti-trichomonal activity of standards and semi-synthesized compounds. Results are mean ± SD of three different experiments performed in triplicate.

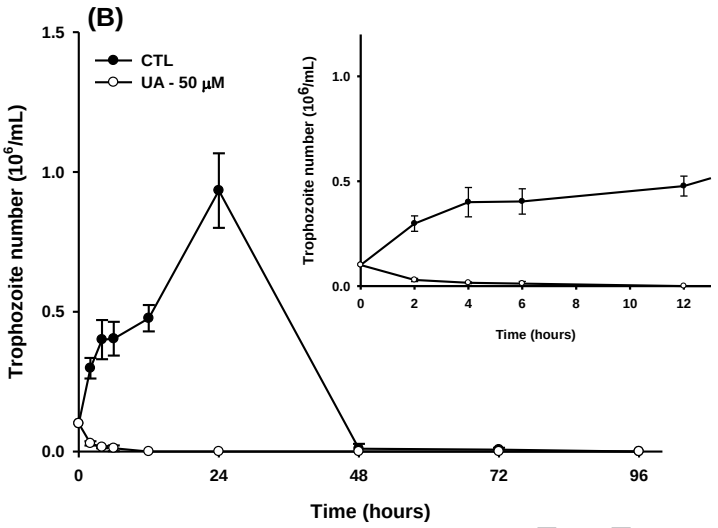
Compounds	***T. vaginalis* viability at 100 µM (mean ± SD)[a]**
Control	100.00 ± 0.00
MTZ	0.00 ± 0.00
α-amyrin (1)	76.10 ± 1.59
β-amyrin (2)	84.30 ± 6.23
Lupeol (3)	117.80 ± 16.80
Oleanolic acid (4)	78.30 ± 8.94
Ursolic acid (5)	0.00 ± 0.00
Acetate of α-amyrin (1a)	56. 40 ± 2.46
Caproate of α-amyrin (1b)	56.20 ± 0.05
Acetate of β-amyrin (2a)	54.50 ± 1.06
Caproate of β-amyrin (2b)	62.80 ± 3.61
Acetate of lupeol (3a)	59.80 ± 4.47
Caproate of lupeol (3b)	71.40 ± 8.36

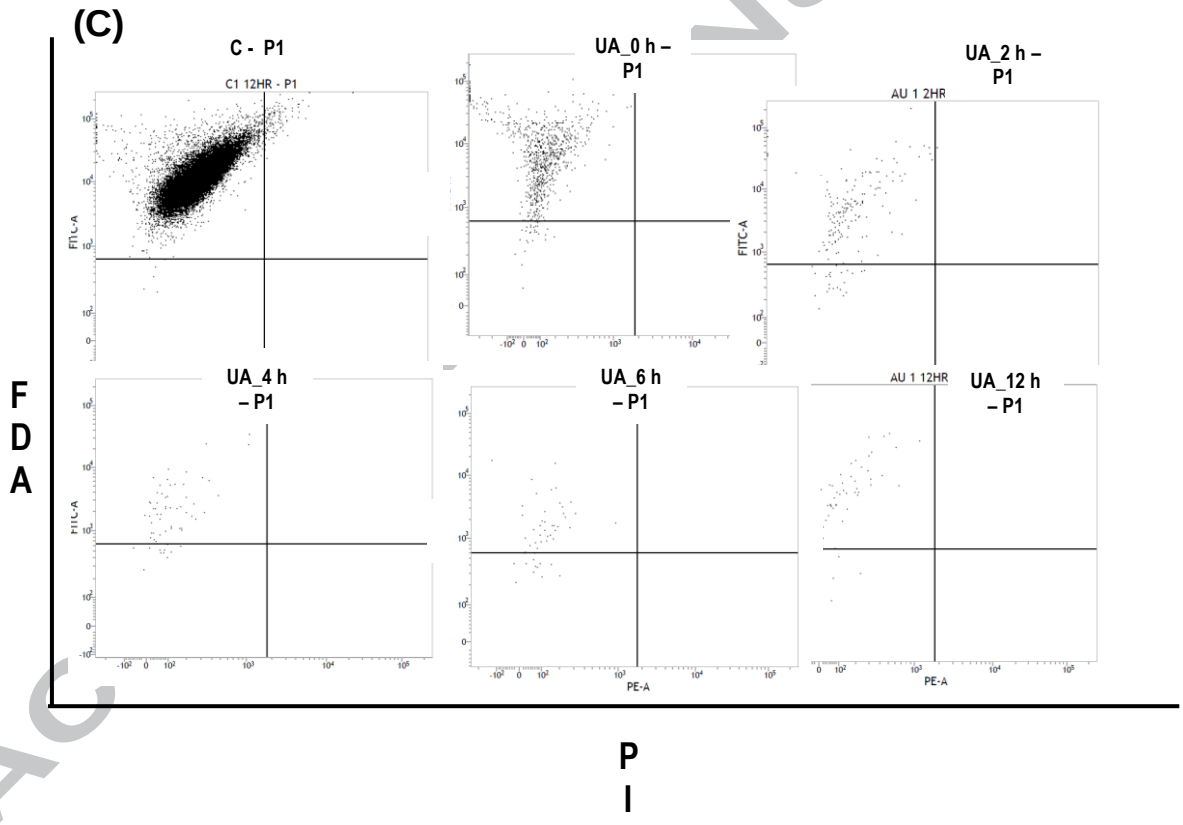
[a]*T. vaginalis* viability was determined by comparison with negative control. MTZ is a positive control.

In contrast, **5** reduced 100% the *T. vaginalis* viability at the same concentration (100 µM) of other evaluated compounds and the MIC was 50 µM (Figure 3A). As demonstrated by Cos et al.[30] pure compounds are considered relevant and selective when present IC_{50}-values below 25 µM. Nevertheless, *T. vaginalis* is an extracellular

organism and an endpoint more robust is required for activity assays; therefore, the anti-*T. vaginalis* activity is usually expressed as MIC-values,[31] because it is necessary a compound that totally abolish the parasite growth, avoiding further growth. In this sense, **5** presented a relevant and selective activity against *T. vaginalis,* demonstrating a potential for new alternative to treat trichomoniasis. To overcome subjective counting mistakes of trophozoite counting under microscopy observation, parasites at MIC and one concentration above were inoculated in fresh TYM medium and the viability assessed each 24 h during 144 h. After 24 h of incubation in new medium, the parasites at 50 and 100 µM of compound **5** did not grow, confirming that 50 µM is a real MIC and the effect was antiproliferative and nonreversible, since parasite growth was inhibited after removal of the compound from medium (Figure 3A inset).







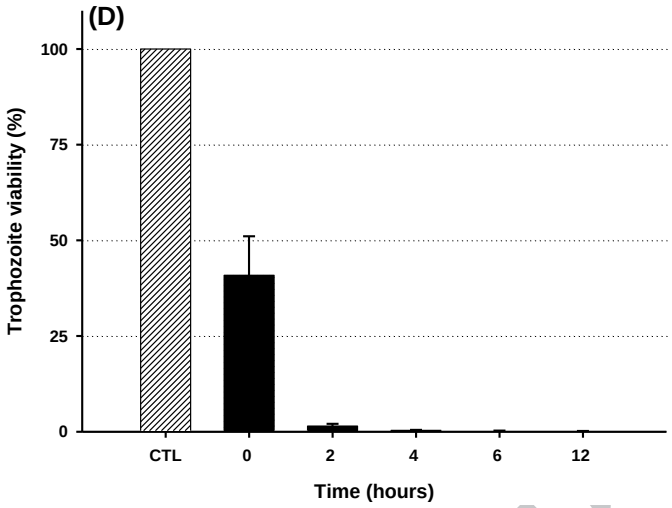


Figure 3.

The effect of **5** on parasite growth and viability was investigated by kinetic growth. After 24 h of incubation, untreated organisms (control) exhibited the classical growth peak. The treated organisms (50 µM of **5**), in turn, did not present the classical growth peak and, after 12 h of incubation, this compound totally abolished parasite growth and only trichomonads debris could be observed (Figure 3B). To increase the accuracy of kinetic growth curve obtained by counting parasites in hemocytometer, flow cytometry assay was performed. The parasites treated or not with ursolic acid were evaluated at different times of incubation (2, 4, 6, and 12 h) and stained simultaneously with FDA and PI. Trophozoites with intact membranes retain FDA dye and were considered as viable organisms. In turn, parasites with compromised membranes incorporate PI dye and were assumed as nonviable. As can be seen in Figure 3C, untreated parasites (control condition) presented only FDA labeling. *Trichomonas vaginalis* treated with **5** exhibited FDA labeling, however, in comparison with control condition (untreated parasites), treated organisms presented much less labeling (Figure 3D). The next experiments performed with **5**, the incubation time chosen was 2

h, because the organisms were burst and only debris was found in times longer than 2 h.

Furthermore, to evaluate morphological and ultrastructural effects of **5** on *T. vaginalis,* trophozoites were treated (or not, as control) with this compound and analyzed by SEM, as shown in Figure 4. Untreated trophozoites presented a typical shape, the teardrop morphology (Figure 4A). After 2 h of treatment with **5**, drastic effects on parasite membrane were observed (Figure 4B-D). Firstly, the typical shape was disrupted and trophozoites became rounded (Figure 4B). Next, it was observed membrane projections and holes. In addition, undulating membrane and flagella was displayed (Figure 4C-D). As explained before, the treatment with **5** destroyed the trophozoites and incubation times longer than 2 h was not possible. SEM assay of *T. vaginalis* demonstrated that **5** treatment caused critical alterations, such as the appearance of rounded and wrinkled trophozoites and significant membrane damage. These features indicate that the parasite is in process of death, and have been previously reported for trichomonads treated with other compounds, such lycorine and candimine,[32,33] and hydroxiquinuclidine derivative.[34]

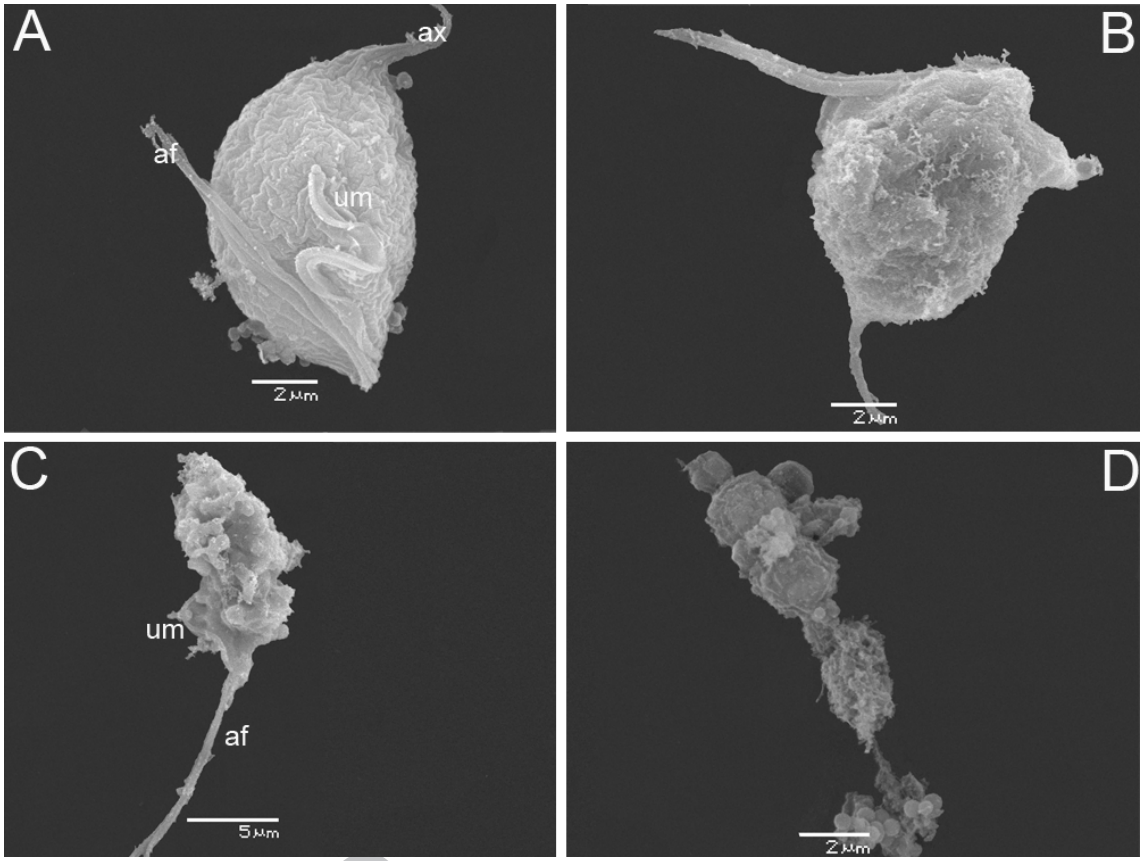
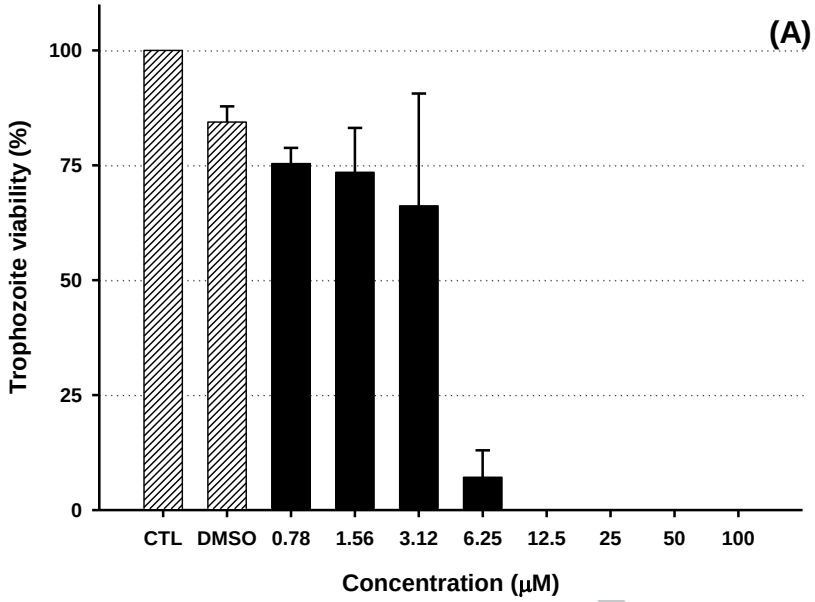


Figure 4.

Taking into account that treatment constitutes a major therapeutic challenge, because approximately 10% of *T. vaginalis* clinical isolates present some level of resistance to metronidazole,[4] new alternatives to treat resistant isolates were necessary. The TV-LACM2R isolate is resistant to metronidazole (MIC is 73 µM)[35] and low concentration of metronidazole (15 µM) reduced about 80% of trophozoite viability. Compound **5** was able to totally reduce TV-LACM2R viability at 12.5 µM (Figure 5A), demonstrating a great potential of this natural derivative against resistant *T. vaginalis* isolates. Furthermore, **5** was evaluated with 15 µM of metronidazole and at this concentration, the compound significantly improved metronidazole effect against TV-LACM2R isolate, indicating a successful synergic effect (Figure 5B).



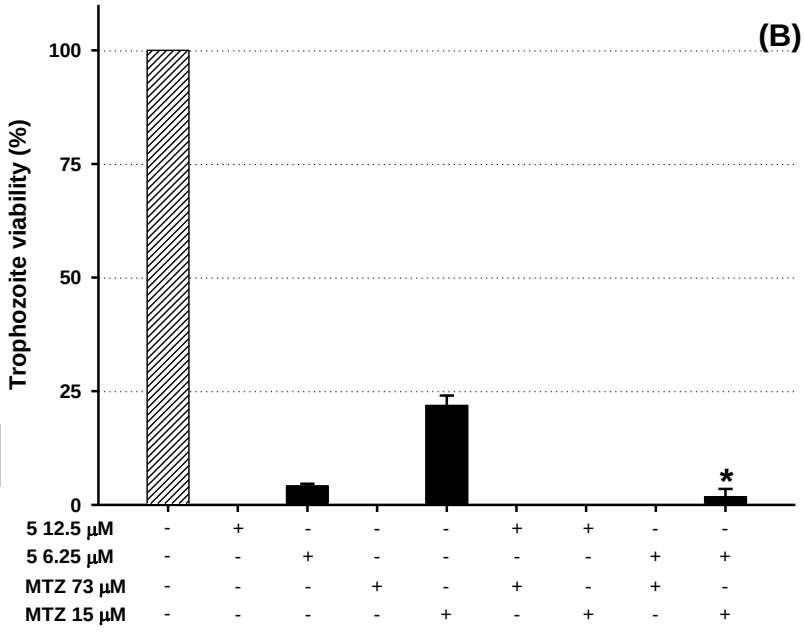


Figure 5

Taking into account the potential of **5** against *T. vaginalis* sensitive and resistant to metronidazole isolates, the *in vitro* cytotoxicity of this compound was evaluated by MTT assay against HMVII and HeLa cancer cell lines and Vero normal cell line. As

showed in Figure 6, after 24 h of incubation, **5** at 50 µM reduced cancer cell lines viability about 60 and 50%, respectively. After 48 h of incubation, the compound showed higher toxicity to these cells, reducing about 90% of cell viability. These findings agreed with earlier studies that demonstrated a great potential of **5** and derivatives against cancer.[28] Furthermore, the viability of Vero cells, normal cell line, was performed and about 30% of cell viability was reduced. In order to compare the activity anti-parasite versus cytotoxicity, the selectivity index (SI) of **5** was calculated as the ratio of cytotoxicity to anti-parasite activity (SI = CC_{50} cell/IC_{50} *T. vaginalis*). The effectiveness of a compound is indicated by selectivity indices ≥10.[36] Thus, low SI indicates high *in vitro* cytotoxicity of the compound and the lack of selectivity for the parasites.

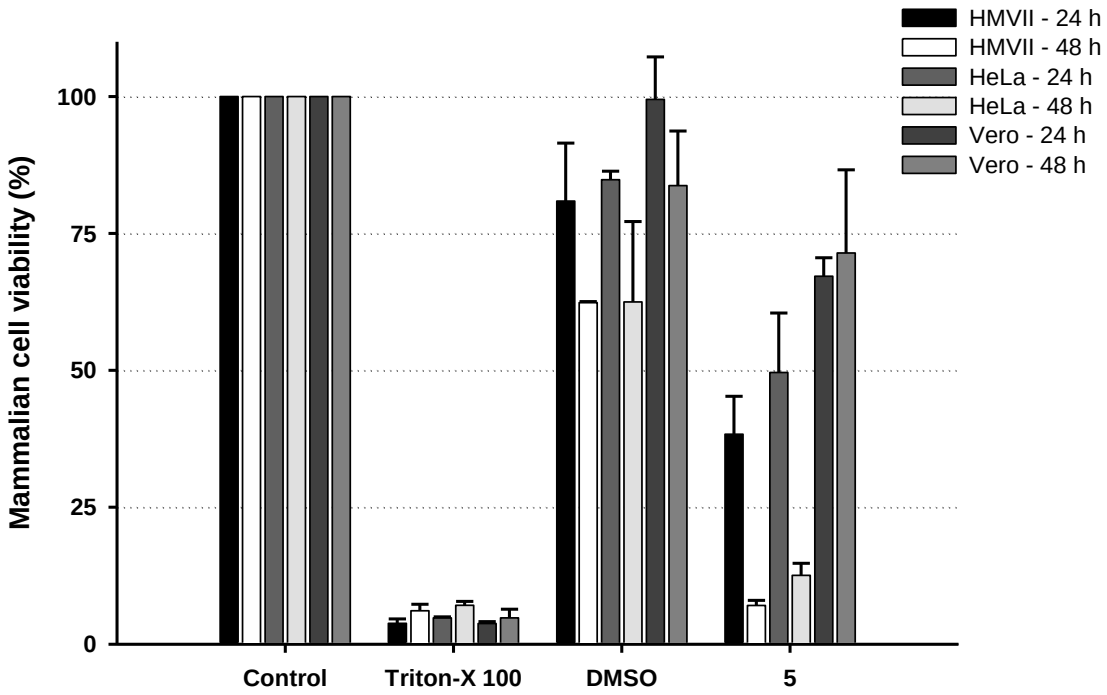


Figure 6

Despite **5** demonstrated a promising anti-*T. vaginalis* activity, this compound presented a low SI against cancer and normal cell lines (Table 2), thereby, showing not

to be selective. Nevertheless, a compound that displayed a high cytotoxicity against mammalian cells should not be abandoned. It is important to emphasize however, that selectivity indices are important for general orientation, but must not be the unique criterion to decide whether a compound should be left aside or forwarded to animal model to continue the search for a new bioactive molecule. As demonstrated in different studies, some compounds showed very favorable SI *in vitro* assays, however, come out to be inactive or very toxic to animals.[37-39]

Table 2. Comparison of anti-*T. vaginalis* activity and mammalian cells effects. Results are representative of three independent experiment.

Condition	**MIC (μM)**	**IC_{50} (μM)**	**CC_{50} (μM)**				**SI**[a]			
Ursolic acid	50	35.3	**HMVII**		**Vero**		**HMVII**		**Vero**	
			24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
			145.9	69.5	31.9	35.9	4.13	1.97	0.90	1.02

[a]SI – selectivity index.

In conclusion, herein we demonstrated the potential of *M. rufula* from Caatinga region against *T. vaginalis.* Collectively, this study provides *in vitro* evidence that **5** is a potential alternative therapeutic choice for treating this pathogen. This compound is a natural compound that can be obtained from a byproduct from juice industry in large and cheap scale and can be used as prototype to development of new derivatives even more potent.

Acknowledgement

This work was supported by the NANOBIOTEC-Brazil program from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil

(grant 23038.019022/2009-68) to AJM, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil (grant 474930/2012-2) to T.T, and FAPESP (Process: 2009/54098-6 and 2012/18031-7) to NPL and DBS. Patrícia B. Vieira receives scholarship from CAPES; Alexandre J. Macedo (grant 305951/2012-2) and Tiana Tasca (grant 307447/2014-6) are supported by researcher fellowships from CNPq. The authors thank Centro de Microscopia Eletrônica (CME/UFRGS) for technical assistance in electron microscopy.

References and notes

1. WHO, Global incidence and prevalence of selected curable sexually transmitted infections: 2008: World Health Organization, Department of Reproductive Health and Research, 2012 ISBN 978 92 4 150383 9.

2. Sutcliffe, S.; Neace, C.; Magnuson, N. S.; Reeves, R., Alderete, J. F., Trichomonosis, a common curable STI, and prostate carcinogenesis - a proposed molecular mechanism, PLoS Pathog., 2012, 8, e1002801.

3. Poole, D. N., McClelland, R. S., Global epidemiology of *Trichomonas vaginalis*, Sex. Transm. Infect., 2013, 89, 418.

4. Schwebke, J. R., Barrientes, F. J., Prevalence of *Trichomonas vaginalis* isolates with resistance to metronidazole and tinidazole, Antimicrob. Agents Chemother., 2006, 50, 4209.

5. Salatino, A.; Salatino, M. L. F., Negri, G., Traditional uses, chemistry and pharmacology of *Croton* species (Euphorbiaceae), J. Brazil Chem. Soc., 2007, 18, 11.

6. Fernandes, C. P.; Correa, A. L.; Lobo, J. F.; Caramel, O. P.; de Almeida, F. B.; Castro, E. S.; Souza, K. F.; Burth, P.; Amorim, L. M.; Santos, M. G.; Ferreira, J. L.; Falcao, D. Q.; Carvalho, J. C., Rocha, L., Triterpene esters and biological activities from edible fruits of *Manilkara subsericea* (Mart.) Dubard, Sapotaceae, Biomed Res. Int., 2013, 2013, 280810.

7. Desrivot, J.; Waikedre, J.; Cabalion, P.; Herrenknecht, C.; Bories, C.; Hocquemiller, R., Fournet, A., Antiparasitic activity of some New Caledonian medicinal plants, J. Ethnopharmacol., 2007, 112, 7.

8. Rhourri-Frih, B.; Renimel, I.; Chaimbault, P.; André, P.; Herbette, G., Lafosse, M., Pentacyclic triterpenes from *Manilkara bidentata* resin. Isolation, identification and biological properties, Fitoterapia, 2013, 88, 101.

9. Shai, L. J.; McGaw, L. J.; Aderogba, M. A.; Mdee, L. K., Eloff, J. N., Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves, J. Ethnopharmacol., 2008, 119, 238.

10. Innocente, A. M.; Vieira, P. B.; Frasson, A. P.; Casanova, B. B.; Gosmann, G.; Gnoatto, S. C., Tasca, T., Anti-*Trichomonas vaginalis* activity from triterpenoid derivatives, Parasitol. Res., 2014, 113, 2933.

11. Silva, G. N.; Maria, N. R.; Schuck, D. C.; Cruz, L. N.; de Moraes, M. S.; Nakabashi, M.; Graebin, C.; Gosmann, G.; Garcia, C. R., Gnoatto, S. C., Two series of new semisynthetic triterpene derivatives: differences in anti-malarial activity, cytotoxicity and mechanism of action, Malar. J., 2013, 12, 89.

12. Guimaraes, A. G.; Serafini, M. R., Quintans-Junior, L. J., Terpenes and derivatives as a new perspective for pain treatment: a patent review, Expert Opin. Ther. Pat., 2014, 24, 243.

13. The general VLC procedure consisted in the use of a silica gel 60 A (6−35 μm) column with a height of 4.0 cm and a variable diameter according to the amount of sample (2.5 cm for 1000 mg; 1.5 cm for 100 mg). Compounds were eluted using *n*-hexane gradually enriched with ethyl acetate (EtOAc) and then EtOAc gradually enriched with methanol (MeOH) reaching a maximum concentration of 15%. Fractions of 15 mL were collected, monitored by TLC (silica gel 60 F254 plates and anisaldehyde-sulphuric acid's reagent were used) and combined according to their profiles.

14. α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) derivatives were synthesized by the addition of the appropriate anhydride (0.032 mmol, 9 Eq) and DMAP (0.021 mmol, 2 Eq) to **1**, **2** or **3** (0.011 mmol) in dichloromethane (1.0 mL) and reacted for three hours without refluxing. Column chromatography of formed product was performed to give the expected pure compounds. TLC was performed on silica gel 60 F254 plates (Merck) and anisaldehyde-sulphuric acid's reagent was used.

15. Data for *3-O-Acetyl-α-amirine* (compound **1a**): white solid. Yield 71%. IR (ATR cm^{-1}): 2961 (C-H); 1733 (C=O); 1447 (C-O); 1241 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ): 0.73 (s, 6H, H_{26} and H_{28}); 0.81 (s, 9H, H_{23}, H_{24} and H_{25}); 0.85 (s, 3H, H_{30}); 0.91 (s, 3H H_{29}); 0.94 (s, 3H, H_{27}); 1.00 (t, 2H, H_1); 1.19 (t, 2H, H_{16}); 1.22 (t, 2H, H_{15}); 1.25 (t, 2H, H_{22}); 1.30 (t, 1H, H_{20}); 1.45 (t, 2H, H_7); 1.98 (s, 3H, H_{32}); 4.44 (dd, 1H, H_3); 5.06 (t, 1H, H_{12}). ^{13}C-NMR ($CDCl_3$,δ): 14.73 (C-25); 15.73 (C-26); 15.85 (C-29); 16.50 (C-6); 17.23 (C-30); 22.22 (C-32); 22.32 (C-24); 22.36 (C-11); 22.39 (C-23); 22.59 (C-2); 25.59 (C-27); 27.06 (C-28); 27.74 (C-15); 30.23 (C-21); 31.85 (C-16); 32.70 (C-7); 35.67 (C-17); 36.68 (C-20); 37.44 (C-10); 37.45 (C-4); 38.59 (C-1); 38.63 (C-19); 39.01 (C-22); 40.52 (C-8); 41.06 (C-14); 46.52 (C-9); 54.14 (C-5); 58.08 (C-18); 79.88 (C-3); 123.54 (C-12); 146.62 (C-13); 171.12 (C-31). HR-ESI-MS *m/z*: 491.3849 $[M+Na]^+$ (calcd. for $C_{32}H_{52}O_2Na^+$ 491.3865, error 3.2 ppm).

16. Data for *3-O-Caproyl-α-amirine* (compound **1b**): white solid. Yield 77%. IR (ATR cm^{-1}): 2929 (C-H); 1729 (C=O); 1450 (C-O); 1245 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ): 0.73 (t, 6H, H_{28} and H_{36}); 0.76 (m, 1H, H_{19}); 0.80 (s, 9H, H_{23}, H_{24} and H_{25}); 0.83 (s, 3H, H_{26}); 0.84 (d, 3H, H_{30}); 0.91 (d, 3H, H_{29}); 0.94 (s, 3H, H_{27}); 1.18 (t, 1H, H_5); 1.25 (m, 8H, H_1, H_{16}, H_{21} and H_{34}); 1.30 (t, 1H, H_{20}); 1.48 (t, 2H, H_{15}); 1.56 (t, 2H, H_6); 1.57 (t, 2H, H_7); 1.75 (d, 1H, H_{18}); 1.84 (t, 2H, H_{33}); 1.97 (t, 2H, H_2); 1.84 (t, 2H, H_{11}); 2.29 (t, 1H, H_9); 2.42 (t, 2H, H_{32}); 4.43 (dd, 1H, H_3); 5.06 (t, 1H, H_{12}). ^{13}C-NMR ($CDCl_3$, δ): 15.68 (C-36); 17.49 (C-26); 18.57 (C-29); 18.62 (C-6); 19.24 (C-30); 19.96 (C-34); 23.16 (C-23 and C-24); 24.06 (C-11); 24.96 (C-2); 25.13 (C-27); 25.39 (C-33); 26.58 (C-28); 28.36 (C-15); 29.80 (C-21); 30.50 (C-34); 33.00 (C-16); 33.10 (C-7); 34.61 (C-32); 35.46 (C-17); 36.61 (C-20); 38.61 (C-10); 39.47 (C-4); 40.13 (C-1); 41.36 (C-19); 41.74 (C-22); 43.26 (C-8); 43.82 (C-14); 49.32 (C-9); 57.01 (C-5); 60.67 (C-18); 82.25 (C-3); 126.07 (C-12); 149.48 (C-13); 159.69 (C-31). HR-ESI-MS *m/z*: 547.4438 $[M+Na]^+$ (calcd. for $C_{36}H_{60}O_2Na^+$ 547.4491 error 9.7 ppm).

17. Data for *3-O-Acetyl-β-amirine* (compound **2a**): white solid. Yield 88%. IR (ATR cm^{-1}): 2947 (C-H); 1729 (C=O); 1462 (C-O); 1247 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ): 0.83 (s, 3H, H_{28}); 0.87 (s, 12H, H_{23}, H_{24}, H_{29} and H_{30}); 0.96 (s, 6H, H_{25} and H_{27}); 1.13 (t, 3H, H_5 and H_{16}); 1.25 (d, 2H, H_{19}); 1.33 (t, 2H, H_1); 1.41 (t, 2H, H_7); 1.52 (t, 2H, H_{15}); 1.58 (t, 4H, H_6 and H_{22}); 1.62 (t, 4H, H_{11} and H_{21}); 1.86 (t, 2H, H_2); 1.96 (t, 2H, H_9 and H_{18}); 2.05 (s, 3H, H_{32}); 4.50 (dd, 1H, H_3); 5.18 (t, 1H, H_{12}). ^{13}C-NMR ($CDCl_3$, δ): 15.70 (C-25); 16.84 (C-26); 16.94 (C-6); 18.40 (C-32); 21.67 (C-23 and C-24); 23.67 (C-11); 23.82 (C-2); 26.10 (C-27); 26.27 (C-28); 27.05 (C-15); 28.18 (C-29); 28.54 (C-30); 31.23 (C-16); 32.63 (C-20); 32.72 (C-21); 33.48 (C-17); 34.87 (C-7); 36.98 (C-10); 37.31 (C-4); 37.86 (C-1); 38.39 (C-8); 39.94 (C-22); 41.84 (C-14); 46.92 (C-19); 47.36 (C-18); 47.70 (C-9); 55.39 (C-5); 81.29 (C-3); 121.86 (C-12); 140.65 (C-13); 176.82 (C-31). HR-ESI-MS *m/z*: 469.4004 $[M+H]^+$ (calcd. for $C_{32}H_{53}O_2^+$ 469.4040, error 7.7 ppm).

18. Data for *3-O-Caproyl-β-amirine* (compound **2b**): white solid. Yield 75%. IR (ATR cm^{-1}): 2917 (C-H); 1723 (C=O); 1454 (C-O); 1248 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ): 0.91 (m, 12H, H_{25}, H_{26}, H_{28} and H_{36}); 0.97 (s, 6H,H_{29} and H_{30}); 1.13 (s, 6H, H_{23} and H_{24}); 1.33 (s, 14H, H_1, H_5, H_7 H_{16}, H_{27}, H_{34} and H_{35}); 1.64 (t, 10H, H_2, H_{19}, H_{21}, H_{22} and H_{33}); 1.86 (t, 2H, H_{11}); 1.95 (t, 1H, H_{18}); 2.36 (t, 3H, H_{32}); 4.49 (dd, 1H, H_3); 5.17 (t, 1H, H_{12}). ^{13}C-NMR ($CDCl_3$, δ): 14.00 (C-36); 15.56 (C-25); 18.31 (C-26); 19.69 (C-6); 22.43 (C-35); 23.61 (C-11); 24.69 (C-23, C-24, C-27 and C-33); 25.97 (C-2); 26.14 (C-28); 26.93 (C-30); 28.05 (C-15); 28.41 (C-29); 31.37 (C-16, C-17, C-22 and 34); 33.34 (C-7); 34.74 (C-21 and C-32); 37.71 (C-1); 39.93 (C-4, C-8 and C-10); 41.62 (C-14); 46.71 (C-19); 47.21 (C-18); 47.51 (C-9); 55.16 (C-5); 80.46 (C-3); 121.60 (C-12); 141.75 (C-13); 179.65 (C-31). HR-ESI-MS *m/z*: 547.4509 $[M+Na]^+$ (calcd. for $C_{36}H_{60}O_2Na^+$ 547.4491 error 3.3 ppm).

19. Data for *3-O-Acetyl-lupeol* (compound **3a**): white solid. Yield 82%. IR (ATR cm^{-1}): 2940 (C-H); 1732 (C=O); 1635 (C=C); 1472 (C-O); 1243 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ):

0.72 (t, 1H, H_5); 0.78 (s, 9H, H_{25}, H_{26} and H_{28}); 0.87 (s, 3H, H_{27}); 0.91 (s, 3H, H_{23}); 0.96 (s 3H, H_{24}); 1.11 (m, 2H, H_{12}); 1.14 (t, 1H, H_{18}); 1.19 (t, 4H, H_1 and H_{15}); 1.32 (m, 6H, H_7, H_{16} and H_{21}); 1.41 (m, 2H, H_{11}); 1.54 (m, 2H, H_6); 1.58 (t, 2H, H_{22}); 1.61 (s, 3H, H_{30}); 1.84 (m, 2H, H_2); 1.97 (s, 3H, H_{32}); 2.30 (m, 2H, H_9 and H_{19}); 4.40 (s, 1H, H_3); 4.50 (brs, 1H, H_{29b}); 4.62 (brs, 1H, H_{29a}). ^{13}C-NMR ($CDCl_3$, δ): 14.41 (C-25); 15.93 (C-26); 16.15 (C-27); 16.46 (C-6); 18.00 (C-32); 18.16 (C-30); 19.21 (C-11); 20.90 (C-28); 21.31 (C-23); 23.67 (C-24); 25.05 (C-2); 27.39 (C-12); 27.95 (C-15); 29.79 (C-21); 34.16 (C-7); 35.52 (C-16); 37.04 (C-10); 37.76 (C-13); 38.30 (C-4); 39.96 (C-1); 40.80 (C-22); 42.75 (C-8); 42.96 (C-14); 47.97 (C-19); 48.24(C-18); 50.29 (C-9); 55.10 (C-5); 80.90 (C-3); 109.19 (C-29); 150.75 (C-20); 171.03 (C-31). HR-ESI-MS *m/z*: 491.3847 $[M+Na]^+$ (calcd. for $C_{32}H_{52}O_2Na^+$ 491.3865 error 3.7 ppm).

20. Data for *3-O-Caproyl-lupeol* (compound **3b**): white solid. Yield 100%. IR (ATR cm^{-1}): 2926 (C-H); 1727 (C=O); 1639 (C=C); 1451 (C-O); 1256 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ): 0.72 (t, 1H, H_5); 0.77 (t, 6H, H_{28} and H_{36}); 0.83 (s, 17H, H_{12}, H_{23}, H_{24}, H_{25}, H_{26} and H_{27}); 0.87 (m, 2H, H_{13} and H_{18}); 0.96 (t, 2H, H_{16}); 1.14 (m, 2H, H_{11}); 1.18 (m, 2H, H_{34}); 1.26 (m, 12H, H_1, H_6, H_7, H_{15}, H_{21} and H_{35}); 1.57 (m, 6H, H_2, H_{22} and H_{33}); 1.61 (s, 3H, H_{30}); 2.20 (t, 1H, H_{19}); 2.22 (t, 1H, H_9); 2.28 (t, 2H, H_{32}); 4.40 (s, 1H, H_3); 4.50 (brs, 1H, H_{29b}); 4.62 (brs, 1H, H_{29a}). ^{13}C-NMR ($CDCl_3$, δ): 13.97 (C-36); 14.52 (C-25); 15.97 (C-26); 16.17 (C-27); 16.57 (C-6); 22.42 (C-30); 23.74 (C-11); 24.84 (C-35); 25.01 (C-23 and C-24); 25.09 (C-28); 27.43 (C-2); 27.96 (C-33); 28.96 (C-15); 29.82 (C-12); 31.48 (C-21); 34.20 (C-34); 34.83 (C-32); 35.57 (C-7); 37.08 (C-16); 37.83 (C-10); 38.03 (C-13); 38.36 (C-4); 39.66 (C-1); 40.00 (C-22); 40.84 (C-8); 42.82 (C-14); 42.94 (C-17); 48.01 (C-19); 48.23 (C-18); 50.27 (C-9); 55.37 (C-5); 80.44 (C-3); 108.98 (C-29); 150.82 (C-20); 179.27 (C-31). HR-ESI-MS *m/z*: 1049.9237 $[2M+H]^+$ (calcd. for $C_{72}H_{121}O_4^+$ 1049.9259 error 2.1 ppm).

21. Mahato, S. B., Kundu, A. P., 13C NMR Spectra of pentacyclic triterpenoids—a compilation and some salient features, Phytochemistry, 1994, 37, 1517.

22. Diamond, L. S., The establishment of various trichomonads of animals and man in axenic cultures, J. Parasitol., 1957, 43, 488.

23. Parasites with a cellular density 1.0 x 10^5 trophozoites/mL were treated with crude extract and fractions (1.0 mg/mL) or semi-synthesized compounds (100 µM). Three controls were carried out: negative control (parasites only), DMSO 0.62% (vehicle for solubilization), and positive control (100 µM metronidazole, Sigma-Aldrich, St. Louis, MO, USA). After 24 h of incubation at 37 °C and 5.0 % CO_2, the number of viable parasites was accessed by counting using a hemocytometer. The results were expressed as the percentage of trophozoite viability compared with untreated parasites after 24 h of incubation, considering motility, normal morphology and exclusion by trypan blue dye (0.2%).

24. After MIC confirmation, parasites in a cellular density of 1.0 x 10^5 trophozoites/mL were treated or not with compound **5** at MIC value (50 µM) and incubated for 2, 4, 6, 12, 24, 48, 72, and 96 h. The parasite number was determined by counting in hemocytometer. The results were expressed as trophozoite number per milliliter comparing with negative control (untreated organisms).

25. In a cellular density of 1.0 x 10^5 trophozoites/mL parasites were treated or not with compound **5** at 50 µM for 4 h. Organisms were harvested and fixed in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2); post-fixed in 1.0% OsO_4 in 0.1 M sodium cacodylate buffer (pH 7.2), and dehydrated in acetone. Critical-point-dried with CO_2 and coated with gold-palladium. The samples were examined with a JEOL 6060 scanning electron microscope.

26. The cell lineages HMVII (vaginal epithelial melanoma cell), HeLa (cervical cancer cell), and VERO (kidney epithelial cells) were grown and maintained in RPMI and

DMEM medium, supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2. For this assay, 3.0 x 10^4 or 1.0 x 10^4 cells per well were seeded in 96-well microtiter plates overnight. After this period, the medium was replaced with fresh medium containing or not (control condition) compound **5** at 50 µM. Positive control (0.2% Triton X-100) was added. The cytotoxicity was evaluated after 24 or 48 h of incubation. After incubation period, media were suctioned; 0.5 mg/mL MTT solution was added and incubated for 1 h. The solution was removed and the insoluble purple formazan was dissolved in DMSO and read at 570 nm.

27. Shiojima, K.; Arai, Y.; Masuda, K.; Takase, Y.; Ageta, T., Ageta, H., Mass Spectra of Pentacyclic Triterpenoids, Chem. Pharm. Bull., 1992, 40, 1683.

28. Hua, S. X.; Huang, R. Z.; Ye, M. Y.; Pan, Y. M.; Yao, G. Y.; Zhang, Y., Wang, H. S., Design, synthesis and in vitro evaluation of novel ursolic acid derivatives as potential anticancer agents, Eur. J. Med. Chem., 2015, 95, 435.

29. Gauthier, C.; Legault, J.; Girard-Lalancette, K.; Mshvildadze, V., Pichette, A., Haemolytic activity, cytotoxicity and membrane cell permeabilization of semi-synthetic and natural lupane- and oleanane-type saponins, Bioorgan. Med. Chem., 2009, 17, 2002.

30. Cos, P.; Vlietinck, A. J.; Berghe, D. V., Maes, L., Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept', J. Ethnopharmacol., 2006, 106, 290.

31. Vieira, P. B.; Giordani, R. B.; Macedo, A. J., Tasca, T., Natural and synthetic compound anti-*Trichomonas vaginalis*: an update review, Parasitol. Res., 2015, 114, 1249.

32. Giordani, R. B.; Vieira, P. B.; Weizenmann, M.; Rosemberg, D. B.; Souza, A. P.; Bonorino, C.; De Carli, G. A.; Bogo, M. R.; Zuanazzi, J. A., Tasca, T., Lycorine induces

cell death in the amitochondriate parasite, *Trichomonas vaginalis*, via an alternative non-apoptotic death pathway, Phytochemistry, 2011, 72, 645.

33. Giordani, R. B.; Vieira, P. B.; Weizenmann, M.; Rosemberg, D. B.; Souza, A. P.; Bonorino, C.; De Carli, G. A.; Bogo, M. R.; Zuanazzi, J. A., Tasca, T., Candimine-induced cell death of the amitochondriate parasite *Trichomonas vaginalis*, J. Nat. Prod., 2010, 73, 2019.

34. Rocha, D. A.; de Andrade Rosa, I.; Urbina, J. A.; de Souza, W., Benchimol, M., The effect of 3-(biphenyl-4-yl)-3-hydoxyquinuclidine (BPQ-OH) and metronidazole on *Trichomonas vaginalis*: a comparative study, Parasitol. Res., 2014, 113, 2185.

35. Butler, S. E.; Augostini, P., Secor, W. E., *Mycoplasma hominis* infection of *Trichomonas vaginalis* is not associated with metronidazole-resistant trichomoniasis in clinical isolates from the United States, Parasitol. Res., 2010, 107, 1023.

36. Mahlo, S. M.; McGaw, L. J., Eloff, J. N., Antifungal activity and cytotoxicity of isolated compounds from leaves of *Breonadia salicina*, J. Ethnopharmacol., 2013, 148, 909.

37. Schmidt, T. J.; Brun, R.; Willuhn, G.; Khalid, S. A., Anti-trypanosomal activity of helenalin and some structurally related sesquiterpene lactones, Planta Med., 2002, 68, 750.

38. Mohammed, R. S.; Abou Zeid, A. H.; El Hawary, S. S.; Sleem, A. A.; Ashour, W. E., Flavonoid constituents, cytotoxic and antioxidant activities of *Gleditsia triacanthos* L. leaves, Saudi J. Biol. Sci., 2014, 21, 547.

39. Fernandes, C. R.; Turatti, A.; Gouvea, D. R.; Gobbo-Neto, L.; Diniz, A.; Ribeiro-Silva, A.; Lopes, N. P.; Garcia, S. B., The protective role of *Lychnophora ericoides* Mart. (Brazilian arnica) in 1,2-dimethylhydrazine-induced experimental colon carcinogenesis, Nutr. Cancer, 2011, 63, 593.

Figure legends

Figure 1. **(A)** Structure of α-amyrin **(1)**, β-amyrin **(2)**, lupeol **(3)**, oleanolic **(4)** and ursolic **(5)** acids. **(B)** Synthesis of α-amyrin **(1a-b)**, β-amyrin **(2a-b)** and lupeol **(3a-b)** derivatives.

Figure 2. Anti-*T. vaginalis* activity of **(A)** crude extracts at 1.0 mg/mL of *Croton nummularius, Manilkara rufula* and *Senna lechriosperma* leaves and branches. CL – *C. nummularius* leaves; CB – *C. nummularius* branches; ML – *M. rufula* leaves; MB – *M. rufula* branches; SL – *S. lechriosperma* leaves; SB – *S. lechriosperma* branches; **(B)** fractions of *M. rufula* leaves at 1.0 mg/mL. CTL – control condition (trophozoites untreated); MTZ – metronidazole (positive control); DMSO – vehicle control. Bars represent the mean ± SD of three different experiments (parasite suspensions) performed in triplicate.

Figure 3. **(A)** MIC determination of UA against *T. vaginalis* 30236 isolate. Inset: MIC confirmation by counting trophozoite number after incubation on TYM fresh medium. **(B)** Kinetic growth curve of UA (50 µM), treated trophozoite in comparison to control (untreated parasites). **(C)** Dot plots of parasite viability treated or not with UA label with FDA-PI. **(D)** Viability quantification from flow cytometry assy. Results are mean ± SD of three different experiments (parasite suspensions) performed in triplicate.

Figure 4. SEM of *T. vaginalis* under UA treatment 50 µM. **(A)** a typical untreated trophozoite displaying a tear-drop shape, four anterior flagella (af), undulating

membrane (um) and axostyle (ax); **(B-D)** after UA treatment for 2 h trophozoites displayed a profound membrane alterations.

Figure 5. MIC determination of metronidazole against *T. vaginalis* TV-LACM2R isolate **(A)**. Combinatory effect of UA and metronidazole against TV-LACM2R isolate **(B)**. *MTZ and UA association statistically different from 15 µM MTZ treatment. Results are mean ± SD of three different experiments (parasite suspensions) performed in triplicate.

Figure 6. Effect of **5** on the viability of HMVII, HeLa and Vero lines at 50 µM. Compound **5** was highly cytotoxic to HMVII and HeLa lines, while to Vero line it presented low cytotoxicity. Controls represent cells only in medium, without **5**. Results are mean ± SD of three different experiments performed in triplicate.

Graphical abstract