UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
DISCIPLINA DE TRABALHO DE CONCLUSÃO DE CURSO

DERIVADOS TRITERPÊNICOS SINTÉTICOS ANTI-*Trichomonas vaginalis*: UMA ALTERNATIVA PROMISSORA

Fernanda Gobbi de Bitencourt

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Trabalho de conclusão de curso apresentado por **Fernanda Gobbi de Bitencourt** como requisito parcial para obtenção de GRAU DE FARMCÊUTICO.

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“A mente que se abre a uma nova ideia

jamais retorna ao seu tamanho original.”

Albert Einstein

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SYNTHETIC TRITERPENOID DERIVATIVES ANTI-*Trichomonas vaginalis*: A PROMISING ALTERNATIVE

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ABSTRACT

Trichomonas vaginalis is an extracellular protozoan parasite that binds to the epithelium of the human urogenital tract during infection named trichomoniasis. In view of increased resistance to drugs belonging to 5-nitroimidazoles class, new treatment alternatives are urgently needed. In this study, eight triterpenes derivatives were synthetized and evaluated for their *in vitro* anti-*T. vaginalis* activity. Compounds obtained from ursolic acid presented better activity than those from betulinic acid. UA-OXYMA, derivative of ursolic acid presented minimum inhibitory concentration (MIC) of 25 µM. To evaluate the behavior of this compound regarding the anti-*T. vaginalis* potential some tests were made. Screening with different *T. vaginalis* fresh clinical isolates showed that the derivative UA-OXYMA was efficient against all parasites. The growth kinetics curve revealed that the compound inhibited parasites growth after six hours of incubation and it totally abolished the parasites proliferation in 24 hours. Hemolysis tests resulted in low hemolytic activity of compound UA-OXYMA; however another test was conducted to identify whether it was cytotoxic and it proved to be in the concentration of 25 µM. Finally, we demonstrated that the compound acts synergistically with the drug of choice, metronidazole. This report reveals the high potential of the triterpenoid derivative compound UA-OXYMA as trichomonicidal agent.

Keywords: Triterpenes, Anti-*Trichomonas vaginalis* activity, ursolic acid, betulinc acid, synthetic derivatives

1. Introduction

Trichomonas vaginalis is a flagellate protozoan that causes trichomoniasis, the most common non-viral sexually transmitted disease (STD) [1,2]. *T. vaginalis* infects a big fraction of individuals [3] and is responsible for 276 million new cases annually worldwide [4]. Nowadays, in women, about 80% of cases are asymptomatic. In symptomatic women, the most common symptoms are vaginal itching, odor, irritation and pruritus, and abdominal pain [4,5]. The most typical signs are edema, erythema and vaginal discharge. A unique feature of the disease is the "cervices whit strawberry appearance"; this is caused by the presence of small punctuate hemorrhagic spots on the vaginal and cervical mucosa. Despite of, this specific signal is observed in a minority of patients [4]. Among men, over than 75 % are asymptomatic and may not seek treatment, generate a chronic inflammation [6,7]. In symptomatic cases, *T. vaginalis* is identified as the etiological agent of the most cases of nongonococcal and nonclamydial urethritis [6]. Trichomoniasis is associated with important health consequences, including predisposition to cervical and prostate cancer [8,9], adverse pregnancy outcomes [10,11], low-birth-weight infants, increased infertility and susceptibility to HIV seroconversion [2,4,12,13].

The therapy is restricted to the class of 5-nitroimazoles (metronidazole and tinidazole), the only drugs approved for the treatment of this disease by FDA [4,14,15]. Nevertheless, metronidazole-resistance of *T. vaginalis* isolates has been increased, and it was reported as 2.5 to 9.6% of clinical isolates [15]. In addition, tinidazole, the alternative to metronidazole, belongs to the same class and cross resistance may be observed. Besides resistance cases, side effects such as nausea, diarrhea, and abdominal discomfort have been observed, stimulating the search of new anti-*T. vaginalis* compounds [4,14]. In this sense, the development of new drugs is urgently necessary.

Plants are commonly used as sources for new drugs discovery. Natural products are a rich source of active compounds representing a promising alternative for the treatment of trichomoniasis [4]. Natural compounds like betulinic and ursolic acids exhibit a variety of biological activities, including anticancer [4,14,15,16,17], inhibition of human immunodeficiency virus (HIV), antibacterial, anti-inflammatory, antimicrobial potential [14,15,16].

The search for triterpene derivatives is a promising approach for development of drugs with potential anti-trichomonal activity. In this context, the aim of this study was to evaluate anti-*T. vaginalis* activity of betulinic acid and derivatives and ursolic acids derivatives.

2. Materials and methods

2.1.Ursolic and betulinic derivatives synthesis

The compounds tested in this study (Table 1) were synthesized as previously described [18] and are briefly shown in a scheme (Figure 1), and described in four steps. In first stage, we prepared the molecules that were used in the other steps. In a round-bottom flask betulinic (I.) or ursolic (II.) acids (0.91g, 1.99 mmol) in acetone; was added Jones reagent at low temperature (0° Celsius). This reaction was stirreds for one hour at room temperature. Isopropanol was added to the resulting mixture at low temperature (0° Celsius), with stirring by 30 minutes. This mixture was filtered with frit G4 and concentrated in rotaevaporator and purified in chromatography column of silica gel. The result was a white and amorphous powder with 86% of allowance, I.a and II.a. In stage two, 0.150g (0.33 mmol) of this powder was added in a round-botton flask and were solubilized with a mixture of ethanol/pyridine (3:1) in nitrogen atmospheric. Then ethanolic solution at hydroxylamine hydrochloride (0.07g, 4.54 mmol) was added. This mixture keeps reacting for 72 hours. Then it was filtrated to give compound as a white powder in 98 yield. I.b and II.b. In the next step, stage 3, the previously product was solubilized in THF, at nitrogen atmospheric and 0° Celsius. After that, piecemeal aluminum hydrate and lithium hydrate were added and the mix was kept under reflux. After 6 hours NaOH 1N was added in solution and water, letting the reaction occurs for one hour. Then the mixture was extracted with diethyl ether. The concentration and purification were made as stated earlier. The product was a white and amorphous powder, with 62% allowance I.c and II.c. In the last step, ammonium nitrate was solubilized with sulphuric acid at 10-15°C. The reaction stayed by agitation for one hour. Then dichloromethane and betulinic acid were added. This mix was treated with water and the organic phase was separated and washed with a solution of sodium carbonate 4%. The extraction, concentration and purification were made as earlier described. The end result was a crystalline and white powder with 90% allowance, I.d.

2.2.Culture of T. vaginalis

Trichomonas vaginalis ATCC30236 and fresh clinical TV-LACM2R, TV-LACM5, TV-LACM6, TV-LACM11, TV-LACM15, TV-LACM22, TV-LACM24 (from female patients) and TV-LACH4 and TV-LACH6 (from male patients) isolates were used in this study. The fresh clinical isolates were obtained from Laboratório de Análises Clínicas e Toxicológicas,

Faculdade de Farmácia UFRGS, Brazil (project approval by UFRGS Ethical Committee, number 18923). Trichomonads were cultured *in vitro* in trypticase-yeast extract-maltose (TYM) medium, pH 6.0, supplemented with 10 % (v/v) heat-inactivated serum, and incubated at 37 °C [19]. Organisms in the logarithmic phase of growth and exhibiting more than 95 % viability and normal morphology were harvested, centrifuged and re-suspended on new TYM medium for compounds assays. All experiments were performed in triplicate and, at least, with three independent cultures (n=3).

2.3.Anti-T.vaginalis *activity assay*

The activity of eight compounds against *T. vaginalis* was determined *in vitro* in the concentration of 100µM. The stock solution of all compounds were prepared in DMSO. For the assay, 96-microtiter plates were used. Parasites at 1.0 x 10^5 trophozoites/mL (final density) were added and were incubated at 37 °C, CO_2 atmosphere for 24 h. Three controls were carried out: negative control with parasites only, vehicle control (DMSO 0.62 % final concentration), and positive control (100 µM metronidazole). The number of viable trophozoites was accessed by counting parasites in hemocytometer using exclusion dye trypan blue (0.2 %). The results were expressed as the percentage of living organisms compared to untreated parasites, considering motility and normal morphology.

2.4.The minimum inhibitory concentration (MIC)

The MIC value shows the lowest concentration of compound able to kill the trophozoites. MIC was determined for UA-OXYMA because this compound was the only compound able to reduce 100 % parasite viability. After compounds eightfold dilution, parasites at 1.0 x 10^5 trophozoites/mL (final density) were added and were incubated at 37 °C, CO_2 atmosphere for 24 h. The number of viable trophozoites was accessed by counting parasites in hemocytometer using exclusion dye trypan blue (0.2 %). MIC values were confirmed by cultivation of parasites in fresh TYM medium, which were analyzed for five days to confirm no parasite growth.

2.5.Kinetic growth assay

Parasites (30236 isolate) at cellular density of 1.0 x 10^5 trophozoites/mL were treated or not with compound UA-OXYMA at final concentration of 25 µM (MIC) and incubated in TYM medium for 2, 4, 6, 12, 24, and 72 h. The number of parasites was counted with hemocytometer and the results were expressed as trophozoite number per milliliter comparing with negative control (untreated organisms).

2.6.Hemolytic Assay

This assay was performed as described by Rocha et al [20] with some modifications. Fresh human blood was obtained from healthy volunteers. The erythrocytes were washed three times with PBS 1x (pH 7.0) and re-suspended to obtain a 1.0 % (v/v) erythrocytic suspension. The concentration of UA-OXYMA was chosen according to the MIC. Then, erythrocytes (1.0 %) were incubated with the samples at 37 °C for 1, 24 and 48 h. Supernatant absorbance was measured at 540 nm. Results were expressed as hemolysis percentage of each test sample, comparing to 100 % hemolysis that was attributed to hemolytic action of the positive control 0.1 % triton X-100 (positive control). This experiment was carried out in triplicate, and three independent experiments (n=3) were performed.

2.7.Cytotoxicity against HMVII and HeLa cells

The cells HMVII and HeLa, representative of vaginal and cervical epithelial cell, respectively, were used to evaluate the cytotoxicity of UA-OXYMA. HMVII lineage was grown in RPMI-1640 medium, while HeLa cells was maintained in DMEM; both media were supplemented with 10 % fetal bovine serum (FBS) and incubated 37 °C, 5 % CO_2. For the assay, 3.0 x 10^4 or 1.0 x10^4 cell/well was seeded in 96-well microtiter plate overnight. The medium was replaced by fresh medium containing or not (control condition) UA-OXYMA at MIC. Triton X-100 at 0.2 %, final concentration, was added as a positive control and 0.6 % DMSO as vehicle control. The plates were incubated for 24 and 48 h, and then, after wash with PBS and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/mL) was added and incubated for one hour. The plates were washed twice with PBS, and the insoluble purple formazan was dissolved in DMSO. The amount of reduce MTT was measured at 570 nm. The experiment was performed twice (n=2) in the times 24 and 48 h.

2.8. Synergism

The TV-LACM2R metronidazole resistant *T. vaginalis* isolate was used in this assay since it presents a MIC value of 73 μM. Low-level resistance is defined as an anaerobic MIC 30-60 μM, moderate-level resistance as 60-120 μM, and high-level resistance as 235 μM or greater as previously described by Butler et al. [21]. Parasites were treated with 12.5 and 6.25 μM UA-OXYMA in association or not to 15 and 73 μM metronidazole and incubated for 24 h at 37°C and 5.0 % CO_2. The parasite viability was determined as describe in the anti-*T.vaginalis* assay. This test was used to evaluate whether the compound and metronidazole present additive effects against *T. vaginalis*.

3. Results

3.1. Screening

In an attempt to observe the activity of betulinc and ursolic acids and their derivatives (Table 1), a screening was conducted at 100 μM (Figure 2). The activities of these compounds were performed against *T. vaginalis* 30236 isolate. Betulinic acid and its derivatives (AB=O, AB-NH_2, AB-OXIMA) and ursolic acid derivative (AU=O) did not present activity against *T. vaginalis*. The AB-ONO_2 and AU-NH_2 derivatives reduced about 56 and 77 % of parasites viability, respectively. Finally, the compound that showed the best activity against the trophozoites was UA-OXYMA, reducing 100 % of viability. Based on screening results, ursolic acid derivative – UA-OXYMA - was the only compound completely active against *T. vaginalis*. In this sense, MIC value was determined as 25 μM (Figure 3) and used in the following experiments.

3.2.Kinetic growth curve

To understand the effect of UA-OXYMA in *T. vaginalis* growth and viability, a kinetic growth curve was performed (Figure 4). The viability of trophozoites was observed under light microscopy until 72 h of incubation at 25 μM. A control was performed and demonstrated a classical growth peak after 24 h of incubation. UA-OXYMA inhibited parasite growth shortly after 6 h of incubation and totally abolished trophozoite growth in 24 h. The results were confirmed by the counting the number of parasites with hemocytometer.

3.3.Other isolates

The compound UA-OXYMA was tested against *T. vaginalis* fresh clinical isolates from women TV-LACM2R, TV-LACM5, TV-LACM6, TV-LACM11, TV-LACM15, TV-LACM22, TV-LACM24 and from men, TV-LACH4, TV-LACH6 (table 2). All isolates were highly sensitive to UA-OXYMA at the MIC concentration, including TV-LACM2R, a fresh clinical isolate resistant to metronidazole, showing no viable parasites. Due to the TV-LACM2R be the only resistant isolate, a serial dilution was made in order to identify the minimum inhibitory concentration value. The results showed that UA-OXYMA was able to kill all organisms in this isolate in the concentration of 12.5µM. Then, this MIC value was used in the synergism test.

3.4. Cytotoxicity

Figure 5 shows that UA-OXYMA was cytotoxic against HMVII and HeLa lineages (7.07 and 6.68 % of viability in 24 h; 15.85 and 5.32% in 48 h, respectively). Although the sensitivity was similar between the two cells lines, the HeLa cells were more sensitive, with values of cell viability below 10 %.

3.5. Hemolysis

In order to investigate whether the mechanism of death by UA-OXYMA involves membrane damage, hemolytic assay was performed [22]. Results showed that the compound at 25 µM did not promote erythrocyte lysis in one hour, and at 24 h the lyses increased about 25 %, reaching around 40 % in 48 h (Table 3).

3.6. Synergism

Finally, in order to investigate the effect of the association between the ursolic acid derivative, UA-OXYMA, and metronidazole, a synergism assay was performed with the *T. vaginalis* TV-LACM2R isolate, resistant to metronidazole (Figure 6). The additive effect between UA-OXYMA and metronidazole is clear observed when low concentrations were tested, 15 µM metronidazole and half of the MIC UA-OXYMA (6.25 µM). This association

completely abolished the trophozoites viability revealing the strong potential of UA-OXYMA as trichomonicidal agent.

4. Discussion

Trichomoniasis is the most prevalent non-viral STD and resistance of the traditional drugs, such as metronidazole and tinidazole, is increasing [4]. Therefore, development of new drugs becomes essential. In new drug discovery, natural and synthetic compounds play an important role [4]. Many studies show that triterpenoid derivatives have good activity against microorganisms; between these compounds, betulinic and ursolic acids have great relevance. In this study, we showed anti-*T. vaginalis* activity of different synthetic derivatives from betulinic and ursolic acids. Although data from literature demonstrate that derivatives from different skeleton have different biological activity [23]. In this study, we demonstrated that derivatives of different skeleton presented similar activities against the parasite.

Ursolic acid is a well-known triterpenoid that has been reported to possess a wide range of biological activities, including antimicrobial, antitumor, antiviral, antiprotozoal, antioxidant, and anti-inflammatory activities [4,14,15,16,17,24]. Among these activities, it showed appreciable anti-parasite effects against *Plasmodium falciparum*, *Toxoplasma gondii*, *Trypanosoma cruzi* and *Leishmania* sp. [25] but has few studies against *T. vaginalis*.

Relevant and selective activity relates to IC_{50}-values below 25 µM for pure compounds [16]. However, anti-*T. vaginalis* activities usually are present by MIC [4]. In this sense, as demonstrated at Figure 3, UA-OXYMA presented MIC of 25 µM, indicating a promising activity against *T. vaginalis.*

In order to elucidate the potential of this compound on growth and viability, a kinetic growth curve was performed. After 6 h of incubation, UA-OXYMA reduced trophozoite viability, however, the completely abolishment of parasite growth appeared after 24 h. This result showed consistent with other studies with different derivatives of this triterpenoid [14].

Taking into account that *T. vaginalis* ATCC and fresh clinical isolates present different characteristics, as virulence and/or pathogenicity, UA-OXYMA was tested against nine fresh clinical isolates TV-LACM2R, TV-LACM5, TV-LACM6, TV-LACM11, TV-LAC15, TV-LACM22, TV-LACM24, TV-LACH4 and TV-LACH6. This test was performed at 25 µM and UA-OXYMA reduced about 100 % of parasite viability of all isolates. This result showed that this compound presents activity against different *T. vaginalis* isolates, showing the potential of UA-OXYMA as new alternative against this pathogen.

Currently resistance to metronidazole is an increasing problem [26] and new alternatives to treat resistant isolates is necessary. Thus, the activity of UA-OXYMA against TV-LACM2R, a *T. vaginalis* metronidazole resistant isolate, was evaluated. This compound presented a MIC of 12.5 µM, which correspond to half of ATCC isolate, demonstrating that UA-OXYMA is an important candidate for designing lead compounds for treat trichomoniasis. In order to evaluate the existence of association between UA-OXYMA and metronidazole, an assay was performed by combining these compounds in different concentration. As showed in figure 6, when 15 µM metronidazole and 6.25 µM UA-OXYMA were tested, a significant reduction of parasite viability was observed, demonstrating that UA-OXYMA is able to potentiate metronidazole effect against a resistant isolate.

Ursolic acid is widely found in various natural products and it exhibits many biological effects including anticancer activity and antimicrobial potential [27]. Studies indicate that the cytotoxic activity of ursolic acid derivatives was also markedly influenced by their structural properties [22,27,28]. In our study we used a semisynthetic ursolic acid derivative and this compound was cytotoxic to two mammalian cell lines, reaching cytotoxicity values close to positive control (Triton-X 100 at 0.2 %), suggesting that structural changes must be made in order to reduce this toxicity to mammalian cells.

In order to investigate the mechanism of death of UA-OXYMA, a hemolytic activity was performed. This compound presented a low hemolytic activity and over the time, the percentage of hemolysis increased, but did not exceed 40 % in 48 h. These results indicated that UA-OXYMA mechanism of death probably does not involve damage in the parasite membrane; however, more studies are needed to elucidate the mechanism of death.

5. Conclusion

Trichomonas vaginalis causes a disease that is a serious public health problem. The increasing resistance to traditional treatments with metronidazole leads us to search for other therapies and a large field of study is natural products, including the synthetic derivatives of natural products. The synthetic triterpene derivative UA-OXYMA demonstrated anti-*T. vaginalis* activity at 25 µM against the ATCC30236 isolate. Furthermore, it showed activity in the same concentration for all the clinical isolates tested. However, this compound was cytotoxic to mammalian cell lines. Therefore, further studies are necessary to perform structural modifications leading to cytotoxicity reduction. Besides that, according the hemolysis assay is possible to suggest that the mechanism of death did not involve the parasite membrane; however

further studies become necessary to provide details regarding the mechanism of death of UA-OXIMA anti-trichomonads activity. Altogether, the results present in this study demonstrated that UA-OXYMA is a promising alternative to treat trichomoniasis.

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Figures caption:

Figure 1. Scheme of synthesis of betulinic and ursolic acids derivatives. **Condition: I. Betulinic acid, II. Ursolic acid**; **I.a AB=O** and **II.a UA=O** (1.99 mmol), acetone, Jones Reagent, 1h, T.a. 86 % of allowance; **I.b AB-OXYMA** and **II.b UA-OXYMA** (0.33 mmol), ethanol/pyridine, hydrochloride hidroxilamona (4.54 mmol), N2(g), 72h, t.a, 98 % of allowance. **I.c AB-NH_2 and II.c UA-NH_2** (0.212 mmol), THF, N2 (g), aluminum and lithium hydride $LiAlH_4$ (1.12 mmol), reflux for 6h t.a, 62 % of allowance. **I.d ABONO$_2$** , ammonium nitrate (1.32 mmol), sulphuric acid, dichloromethane, betulinic acid (0.48 mmol), 1h, 90 % of allowance.

Figure 2. Anti-*T. vaginalis* activity of semisynthesized derivatives of betulinic and ursolic acids against ATCC30236 isolate. This screening was performed at compounds concentrations of 100 µM. Data are presented as mean ± SD compared to control (considering trophozoite viability 100 %).

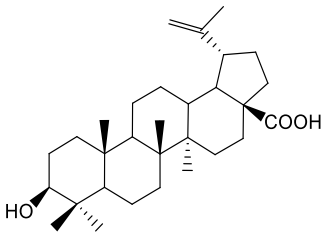
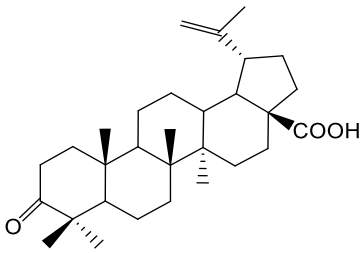
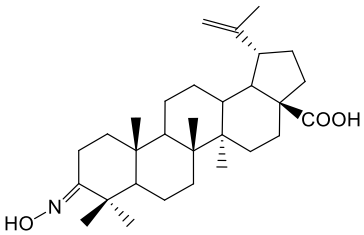
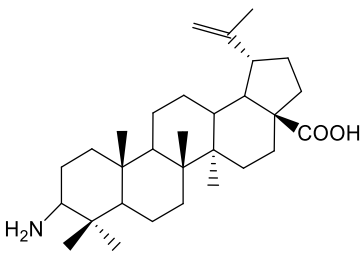
Figure 3. Susceptibility of *T. vaginalis* trophozoites to UA-OXYMA ranging concentration from 100 to 0.78 µM. The value of MIC was established in 25µM.

Figure 4. Kinetic growth curve of UA-OXYMA, represented by dotted line (25 µM) treated trophozoites (ATCC30236 isolate) in comparison to untreated parasites, represented by continues line (control) and parasites with DMSO (vehicle). The initial inoculum was 1.0×10^5 trophozoites/mL. Results are representative of three independent experiments in triplicate.

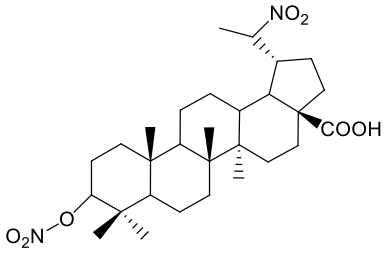
Figure 5. MTT cytotoxic assay of UA-OXYMA using HMVII and HeLa cells. Cells were exposed to 25 µM UA-OXYMA for 24 and 48 h; Triton X-100 was used as positive control; and control means only cells in medium without exposure to UA-OXYMA. Data represent mean ± SD compared to control (only cells).

Figure 6. Synergism of UA-OXYMA with metronidazole, in MIC and MIC/2 of the compound. Data represent mean ± SD compared to control (only trophozoites).

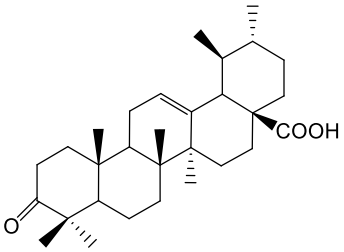
Table 1. Compounds structures.

Name	structure
Betulinic acid **(AB)**	
Betulinic acid rusty **(AB=O)**	
Betulinic acid oxyma **(ABOXYMA)**	
Betulinic acid amines **($ABNH_2$)**	

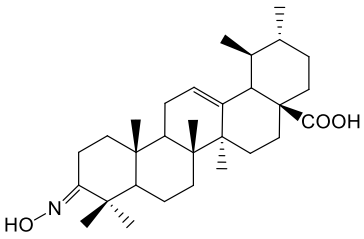
Betulinic acid nitric ester
(ABONO$_2$)



Ursolic acid rusty
(UA=O)



Ursolic acid oxyma
(UAOXYMA)



Ursolic acid amines
(UANH$_2$)

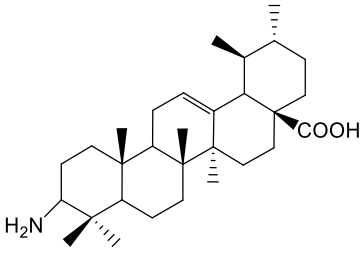


Table 2. Anti-*T. vaginalis* activity of UA-OXYMA.

Trichomonas vaginalis isolates	Trophozoite viability (%) at 25 μM
	UA-OXYMA
TV-LACM2R	0.00 ± 0.00
TV-LACM5	1.03 ± 1.25
TV-LACM6	2.85 ± 2.91
TV-LACM11	0.00 ± 0.00
TV-LACM15	0.00 ± 0.00
TV-LACM22	0.00 ± 0.00
TV-LACM24	0.00 ± 0.00
TV-LACH4	0.00 ± 0.00
TV-LACH6	0.77 ± 1.34

Table 3. Hemolytic activity of UA-OXYMA at 25 µM.

	Hemolysis (%)		
	1 h	24 h	48 h
Positive control	100 ± 0	100 ± 0	100 ± 0
Negative control	5.52 ± 0.9	6.56 ± 0.003	9.71 ± 0.004
UA-OXYMA	5.75 ± 0.81	25.44 ± 0.022	39.10 ± 0.065

Figure 1.

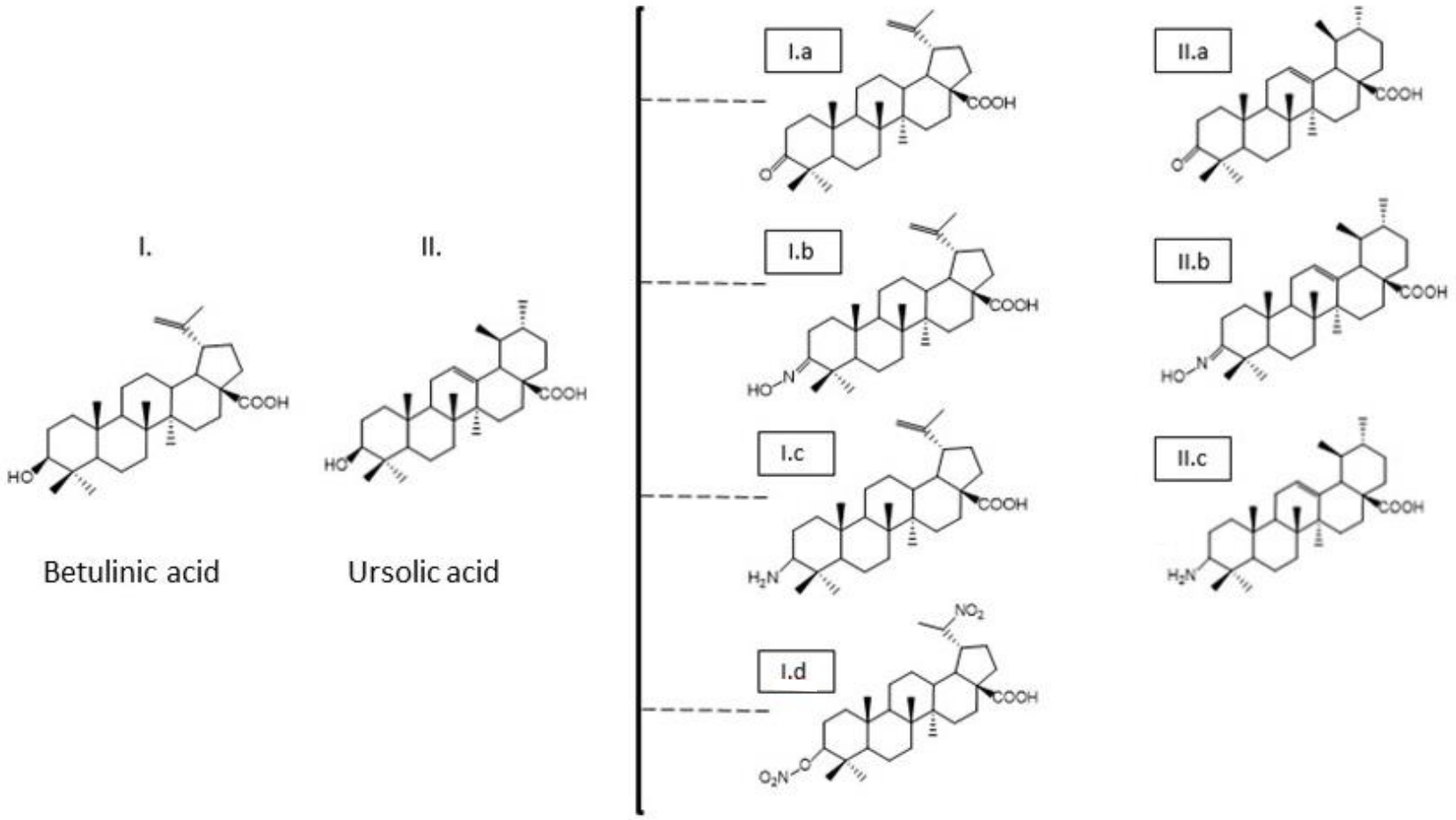

I.
II.
COOH
HO
Betulinic acid
Ursolic acid
I.a
I.b
I.c
I.d
II.a
II.b
II.c
O
HO-N
H2N
NO2
O2N-O

Figure 2.

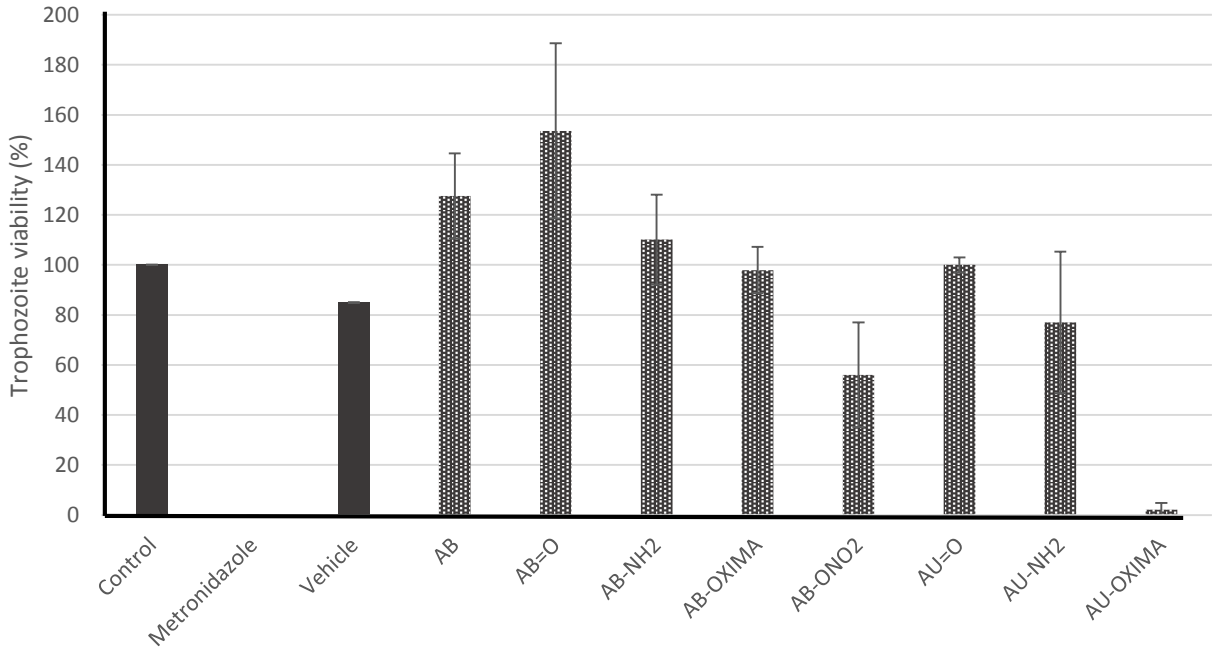

Trophozoite viability (%)
200
180
160
140
120
100
80
60
40
20
0
Control
Metronidazole
Vehicle
AB
AB=O
AB-NH2
AB-OXIMA
AB-ONO2
AU=O
AU-NH2
AU-OXIMA

Figure 3.

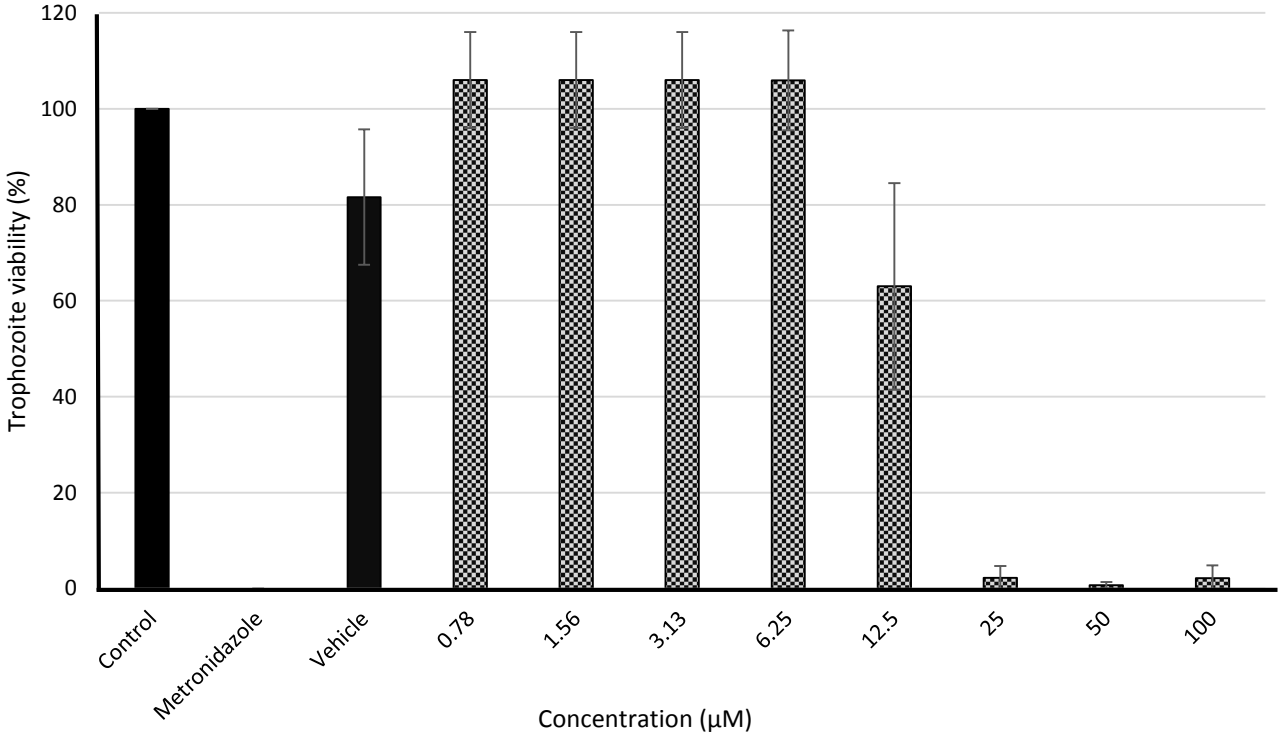

120
100
80
60
40
20
0
Trophozoite viability (%)
Control
Metronidazole
Vehicle
0.78
1.56
3.13
6.25
12.5
25
50
100
Concentration (µM)

Figure 4.

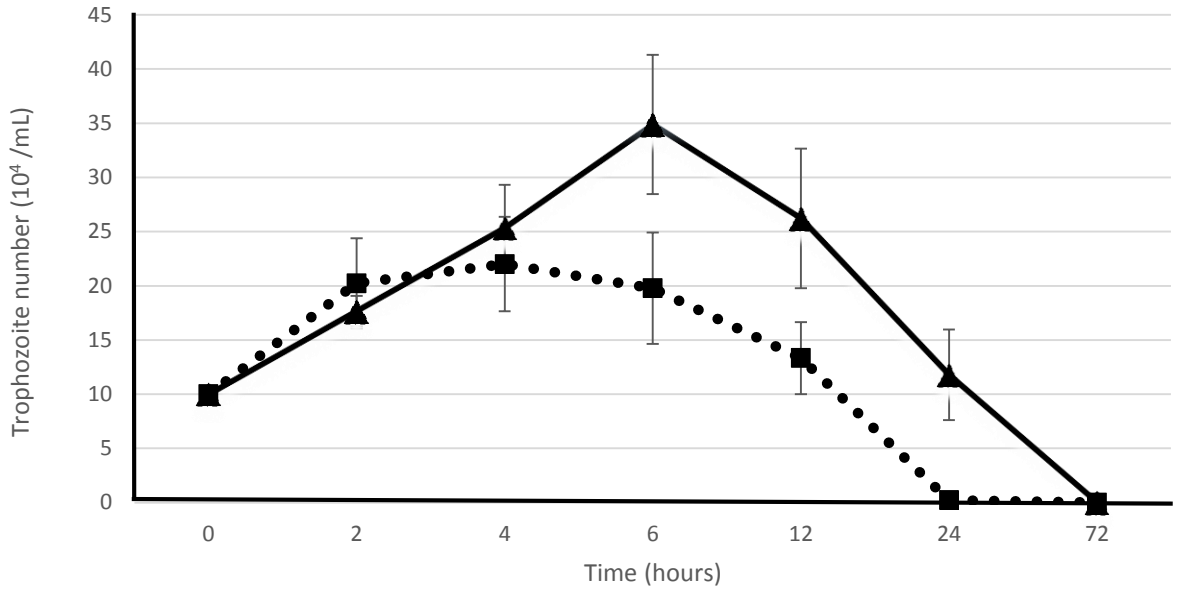

45
40
35
30
25
20
15
10
5
0
Trophozoite number (10^4 /mL)
0
2
4
6
12
24
72
Time (hours)

Figure 5.

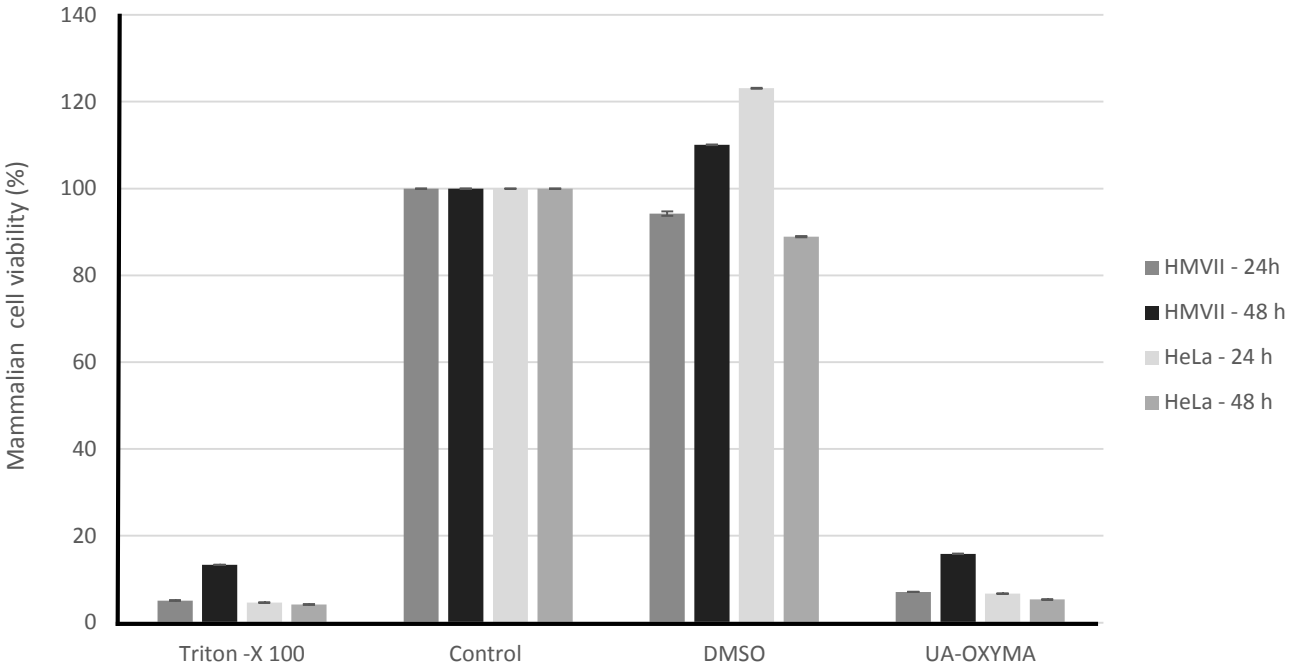
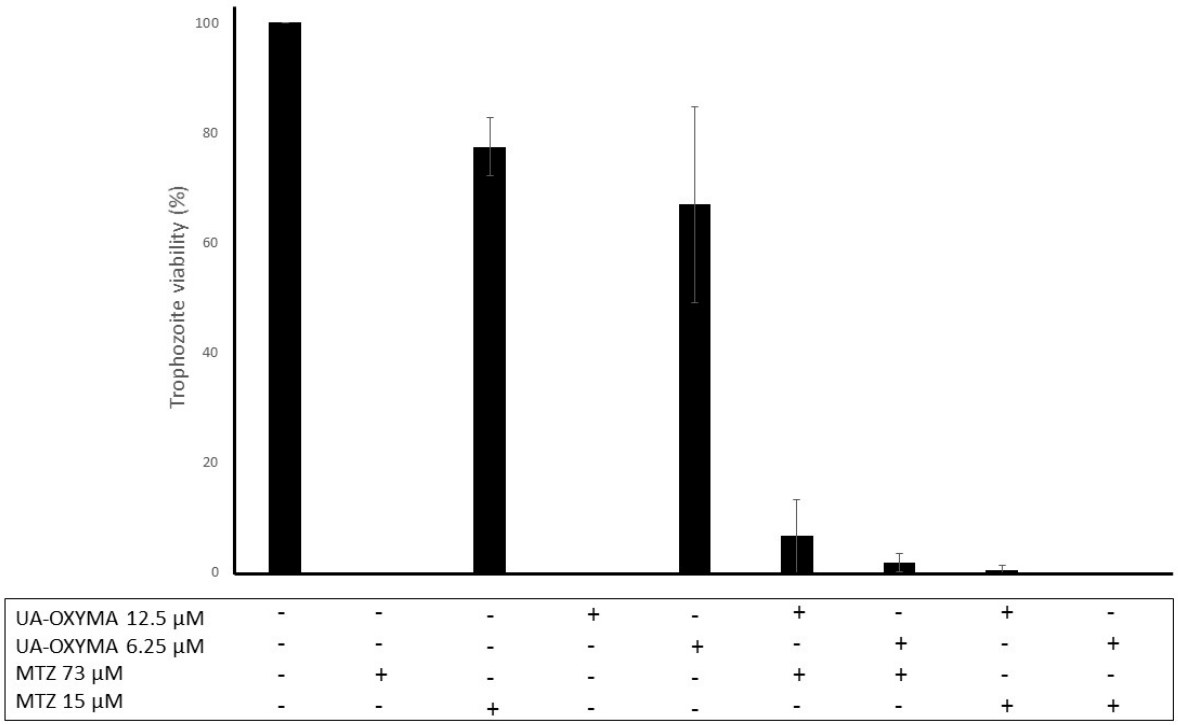

140
120
100
80
60
40
20
0
Mammalian cell viability (%)
Triton -X 100
Control
DMSO
UA-OXYMA
HMVII - 24h
HMVII - 48 h
HeLa - 24 h
HeLa - 48 h

Figure 6.


Trophozoite viability (%)
100
80
60
40
20
0
UA-OXYMA 12.5 µM - - - + - + - + -
UA-OXYMA 6.25 µM - - - - + - + - +
MTZ 73 µM - + - - - + + - -
MTZ 15 µM - - + - - - - + +