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Anti-parasitic activity of nano *Citrullus colocynthis* and nano *Capparis spinose* against *Trichomonas vaginalis in vitro*

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Research Article

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Abstract

The use of plant extracts and the benefit of their unique properties in treating various pathogens is the return to mother nature, and an attempt to overcome the problems of side effects resulting from the use of chemical drugs and the ability of some pathogens to resist these drugs. Nanotechnology has strengthened the ability of drugs to reach the target and reduced the size and amount of dose needed for treatment.

Nano-extracts of Citrullus colocynthis and Capparis spinosa at concentrations of (100,250 and 500) ppm prepared to the treatment *Trichomonas vaginalis* in vitro at the time (12 , 24, 72)h. Results compared with the use of 0.1% of metronidazole (500 mg).

The results showed that the concentrations (100,250, 500) ppm of *C. colocynthis* had an inhibitory activity for the growth rate (43.77, 69.15, 89.89) at the time (12, 24 and 72) hours, respectively. The inhibitory activity of *C. spinosa* was (43.18, 67.41, 87.04) at the same time and concentration, compared with metronidazole (43.47, 70.40, 87.04) at the same time. Neither plants showed severe effects in hemolysis.

From the results, it can be concluded that either plant can be used as an alternative to metronidazole after completing human and animal tests.

Highlights

- Two types of nano plants (*Citrullus colocynthis* and *Capparis spinosa*) have been successfully used to treatment of *Trichomonasvaginalis* in vitro compared with Metronidazole drug .
- All treatments showed moderate results in inhibiting Parasite growth or elimination it, where used medium concentrations from both plants.
- Neither plants showed severe effects in hemolysis.

Introduction

Trichomoniasis is a globally widespread disease that infects both men and women [1]. Although many researchers consider men a carrier of this parasite, women considered as a reservoir host. This disease is associated with infection by many pathogens, the most prominent of which is HIV [2]. It is also related to the emergence of some cases of infertility and the birth of loss-weight children [3].

Trichomoniasis is treated with nitroimidazoles derivatives such as Flagyl (5-Metronidazole) Which results in few side effects, and in any case, there are strains of this parasite that are resistant to the treatment that increases the cases of non-response (Refractory cases) subsequently necessitate the use of high and toxic doses of the Flagyl drug for longer periods[4] and this, in turn, The side effects of nausea, vomiting, dizziness, headaches, rashes, dry mouth, metallic taste, leukopenia, and neuropathy are more

severe [5]. Therefore, attention was drawn to the use of alternative healing methods, which are medicinal herbal treatments, due to their widespread. Numerous studies have indicated that the plantea kingdom is rich in its by-products resulting from the processes Metabolites, which are characterized by having anti-microorganisms such as glycosides, lobules, and other bitter materials, saponins, alkaloids, and alkaloids [6].

Nano-compounds have been widely used in the treatment and diagnosis of many diseases, as they have shown an excellent ability to deliver the drug to the target, in addition to many chemical and physical features of these compounds[7].

The purpose of this study is to test the ability of nano-composites extracted from two common plant species, *Citrullus colocynthis* and *Capparis spinosa* against *Trichomonas vaginalis*. As well as testing the side effects of the use of this type of drugs and their impact in hemolysis.

Materials And Method

Samples collection

Samples were collected from women suspected of having trichomoniasis and visits to private gynecology clinics, with the help of specialized physicians. Samples were collected using a sterile speculum without disinfectant or lubricant to avoid the inhibitory effects of the parasite, as the swabs were taken from the lining of the vagina or from Posterior fornix of the cervix using a sterile cotton swab that was rotated before its withdrawal[8], this swab was soaked for three days in the In pouch culture system (Biomed, Diagnostics, Santa Clara) , California, USA)[9].

2.2 Parasite development

T. vaginalis were grown in Diamond's Medium Tryptone, Yeast, Maltose (TYM) was inoculated by adding 0.1 cm^3 of a parasite-positive culture (free from contamination) at a 3-day age, during the logarithmic phase of growth in Glass bottles containing 4.9 cm^3 of the new culture medium

with an initial number of 1x 10^5 cell / cm^3 and this was done under sterile conditions, after which the bottles were incubated at a temperature 37 °C, and the perpetuation process was repeated every 3 days[8].

2.2.1 **Calculate the number of parasites**The number of *T. vaginalis* in the culture was calculated every 72, 24 and 12 hours using the Hemocytometer (Neubaeur). 0.9 cm^3 of cultured parasites was taken , and 0.1 cm^3 of formalin solution 40% was added , equivalent to one drop to fix parasites during the count. The counting process was carried out using an optical microscope and Methylene blue dye 1%, the growth factor GI was calculated by the following equation:

$$GI\% = \left(1 - \frac{GR\ extract}{GR\ control}\right) \times 100^{10}$$

2.3 Collect plants and prepare the extract

The Pulp of the plants' fruits were collected from the rural areas in Al- Hamzah – Iraq (31° 51 ' N and 45° 3'E the southeast of the capital Baghdad), the dust and dirt attached to them were removed and then preliminary sterilization was performed by immersing them in a solution of a minor diluted at 1% for one minute, after that, they have dried naturally away from sunlight for 4-5 days, and kept in paper envelopes until used in the preparation of the aqueous extracts[11]. The aqueous extracts were prepared by relying on the method [12].

2.4 nano-emulsion of plants

Rahimi et al. (2015) method was used, which is, in brief, mixing the extract with oily minerals and adding to distilled water, stirred to mix well after placing it on a magnetic heater at a temperature of 40 $^{\circ}$C, and the mixture was centrifuged with a force (1000 rpm), the emulsion was cooled by placing it in an ice bath, after which the following concentrations (100, 250, 500) PPm were prepared[13].

2.5 Experience design

Three replicates of the culture medium were used for each concentration of each plant, in addition to three replicates of the positive control to which 500 mg of Metronidazole was added at a concentration of 0.1%, all inoculated with a starting number of 1×10^5 Cell / cm^3 and this was done under sterile conditions, in addition to three replicates (negative control) to which distilled water was added[10].

2.6 Toxicity tests

The toxicity of the nanocomposites was estimated by method of [14] as brief.

Preparation of Erythrocytes: 5 ml of blood are drawn from healthy individuals, placed in tubes containing EDTA, Blood components are separated by a centrifuge at 500 rpm for 10 minutes, components are washed with neutral saline and the supernatant is discarded, prepare of 5% v\v of Erythrocytes suspension with neutral saline.

Evolution of Extracts cytotoxicity: three groups of Erythrocytes suspensions, A- incubate with different concentrations of Plant extracts, B- (negative control) un treated suspension, C- incubate with sterile distilled water (positive control).

By “spectrophotometer at 540 nm, the maximum absorption wave length of heamogobin occur, use microplate reader”, heamolysis evaluate as following equation:

$$\text{Heamolysis \%} = \frac{A-B}{C-B} \text{ X } 100$$

2.7 Statistical analysis

Complete random design (CDR) was used in the analysis of the experiments and statistically tested using the Duncan multiple range tests, to see if there was a significant difference between the treated and non-treated media. (Control) of aqueous extracts according to the concentrations used at a significance level $P \leq 0.05$. By statistical analysis Spss version 24.

Results And Discussion

3.1 Result

Concentrations (100, 250, 500) PPm of the nano composite of *Citrullus colocynthis* were used to demonstrate their effect on T. *vaginalis* compared to non-treatment (control) as shown in Table (1), Figure(1) that indicate a reduction in the numbers of parasite in an inverse relationship with the increase in the different concentrations in the solution. When analyzing the results statistically, it was found that there are significant differences at a probability level $p \leq 0.05$ between the growth rate of treated and untreated samples, as it had a clear toxic effect on growth. The extract concentration that caused inactivation of 50% of parasites within 24 hours of growth (IC50) was 99 ppm Table(3). It was also observed that the percentage of growth inhibition increased from 17.8% to 46.1% when increasing concentrations compared to an inhibition rate (43.4%) when using metronidazole within 12 hours of growth. While the rate of inhibition increased from 43.7% to 69.1% within 24 hours, while metronidazole inhibited growth was 70.4% at the same time. After 72 hours of growth, the inhibition rate increased from 53.4% to .89.8% compared to the drug's inhibition rate of 87%.

Table (2) indicated the effect of concentrations (100, 250, 500) ppm of the nano composite For *Capparis spinosa* fruits on the number of treated parasites compared to untreated. As the increase in concentration led to a decrease in the number of parasites, with an inverse relationship during different growth periods. It was clear that the IC50 concentration of *C. spinosa* fruit extract was 99.4 ppm during the logarithmic phase compared with the control Table (3), as this concentration was used in the subsequent experiments. The results of the statistical analysis showed that there are significant differences at the probability level $p \leq 0.05$ between the rates of the numbers of parasites treated with the concentrations of this extract and the rates of the numbers of untreated (control) during the different growth periods.

Table (2) and Figure(2) indicated that the percentage of inhibition increased from 16.9% to 43.1% when increase the concentrations within 12 hours of growth, while metronidazole had a higher effect (43.4%). The rate of inhibition of metronidazole was higher after 24 hours of growth, reaching 70.4%, while the rate of inhibition using concentrations of plant extract increased from 42.5% to 67.4%. After 72 hours of

growth, the highest inhibition rate was recorded by the nanoscale solution, and it was 87%, which is the same as that of metronidazole.

The extracts used in the experiment showed a weak ability to hemolysis red blood cells. The highest effect of *C. spinose* and *C. colocynthis* was at a concentration of 500 ppm, with a percentage of degradation (2.8%) and (2. 3%), respectively, while the plants showed the lowest percentage of degradation at 100 ppm when it was recorded (0.3%) and (0.1%), respectively figure (3).

3.2 Discussion

The present study indicated the ability of nano composites of *C. colocynthis* to remove *T. vaginalis* in a more effective concentrations than metronidazole, and gradually upon increasing concentration. *C. spinose* was equally effective as metronidazole. On the other hand, both plants showed a slightly toxic in hemolysis.

Metronidazole breaks down DNA by releasing free nitrogen radicals and then the cell will die, as the parasite metabolism depends on the generation of ATP through oxidation of acetate produced by pyruvate and in the presence of Co A, nitrogen in the drug works to capturing an electrons that produced by the transport enzymes chain, thus forming free nitrogen radicals and the cell dies [15]. However, many strains of this parasite existed that were resistant to 5-metronidazole and its derivatives[16] due to the frequent use of this drug [17] or to genetic changes in the parasite [18].

Medicinal plants have been used throughout a person's life and have been accompanying his life path since existence on the earth. It is known that many medicinal plants possess many bio-active ingredients such as phenolic, alkaloids, tannins, terpenes, steroids, saponins, flavonoid compounds[11], but the large size of these particles made it difficult to pass through the lipids of the plasma membrane, thus their effectiveness was weak, and an effective effect could only be obtained with an increase in the concentration of these substances, which causes toxicity to living cells and produces negative effects[19]. Preparation of nanocomposites from these plants solves the large size problem and improves their ability to pass through the plasma membrane by generating nanocomposites surrounded or carried by oil [20]. Also, these compounds become more stable and soluble, which delays the ability to build resistance to these drugs [19].

Many herbal extracts have been used in treating *T. vaginalis* [21,22,23] , and nanocomposites of some plants have also been used in the treatment[10,11]. From these plants *C. colocynthis*. The pulp of the fruits of these plants is used as sedatives and anti-inflammatories [24]. It is recommended to use unripe fruits that do not contain seeds [25]. “The pulp contains a bitter compound called colocynthene, a resin called colocynthin, colocynthia, pectin” [26]. Of the active compounds that *C. colocynthis*, alkaloids, and this substance induces programmed death of parasite cells, changes in morphology, interruption of the cell cycle, and cytoplasmic vacuolization [27]. Terpenes, an example of this is the (Capsaicin) terpene, have high biological activity in humans as it affects the nerves, and is a pain reliever, but it inhibits various

types of pathogens, and its effectiveness against microorganisms is also attributed to its possession of a property. It is lipophilic and can link with the wall of a living cell and influence it through it inside it and work on forming complexes and connections with the components of the cell leading to the weakening of its vitality and destruction [28]. Flavonoids are described as antibiotics, as studies have found that they have effectiveness against bacteria, fungi and viruses [29].

C. spinose also contains many active ingredients such as glycosides, myrosinase, rustic acid, caproic acid, pectic acid, saponin, alkaloid substances such as Stachydrine And flavonoids such as Lerpenes and Flavonoids[30], "Plants can produce a large amount of relatively small organic chemicals that are defined as secondary metabolites that lead to pharmacological or toxic effects"[30]. These compounds act as analgesics and anti-inflammatories, as well as inhibit the growth and reproduction of microorganisms [31]. Also, the pulp contains more flavonoids and phenolic than the roots, which affect energy metabolism and plasma membrane permeability[32], and alkaloids that can do the same action[33].

Conclusions

The nano-extracts of C. *spinose* and *C. colocynthis* are efficient in removing *Trichomonas vaginalis*, this ability increases with increasing concentration, which prevents the parasite from forming a resistance to the drug, and it is also safe and side effects from its use are less than the drug metronidazole.

References

1. Sutton, M. *et al.* The Prevalence of Trichomonas vaginalis Infection among Reproductive-Age Women in the United. **30333**, 2001–2004 (2007).
2. Kissinger, P. & Adamski, A. Trichomoniasis and HIV interactions : a review. **89**, 426–433 (2013).
3. Mann, J. R. & Mcdermott, S. Are maternal genitourinary infection and pre- eclampsia associated with ADHD in school-aged children ? **15**, 20837984 (2020).
4. Kirkcaldy, R. D. *et al.* Trichomonas vaginalis antimicrobial drug resistance in 6 US cities, STD surveillance network, 2009-2010. *Emerg. Infect. Dis.* **18**, 939–943 (2012).
5. Schwebke, J. R. & Barrientes, F. J. Prevalence of Trichomonas vaginalis isolates with resistance to metronidazole and tinidazole. *Antimicrob. Agents Chemother.* **50**, 4209–4210 (2006).
6. Mohanty, S. K., Swamy, M. K., Sinniah, U. R. & Anuradha, M. Leptadenia reticulata (Retz.) Wight & Arn. (Jivanti): Botanical, agronomical, phytochemical, pharmacological, and biotechnological aspects. *Molecules* **22**, 1–27 (2017).
7. Patra, J. K. *et al.* Nano based drug delivery systems: Recent developments and future prospects 10 Technology 1007 Nanotechnology 03 Chemical Sciences 0306 Physical Chemistry (incl. Structural) 03 Chemical Sciences 0303 Macromolecular and Materials Chemistry 11 Medical and He. *J. Nanobiotechnology* **16**, 1–33 (2018).

8. VanderPol, B. Clinical and Laboratory Testing for Trichomonas vaginalis Infection. *J. Clin. Microbiol.* **54**, 7–12 (2016).
9. Patil, M. J., Nagamoti, J. M. & Metgud, S. C. Diagnosis of Trichomonas vaginalis from vaginal specimens by wet mount microscopy, in pouch TV culture system, and PCR. *J. Glob. Infect. Dis.* **4**, 22–25 (2012).
10. Vazini, H. Anti-Trichomonas vaginalis activity of nano Micana cordifolia and Metronidazole: an in vitro study. *J. Parasit. Dis.* **41**, 1034–1039 (2017).
11. Al-Ardi, M. H. The uses of gold nanoparticles and Citrullus colocynthis L. nanoparticles against Giardia lamblia in vivo. *Clin. Epidemiol. Glob. Heal.* **8**, 1282–1286 (2020).
12. Azizi, S., Mohamad, R., Shahri, M. M. & McPhee, D. J. Green microwave-assisted combustion synthesis of zinc oxide nanoparticles with Citrullus colocynthis (L.) schrad: Characterization and biomedical applications. *Molecules* **22**, 1–13 (2017).
13. Rahimi, M. T. *et al.* Scolicidal activity of biosynthesized silver nanoparticles against Echinococcus granulosus protoscolices. *Int. J. Surg.* **19**, 128–133 (2015).
14. Elizondo-Luevano, J. H. *et al.* In vitro effect of methanolic extract of argemone mexicana against trichomonas vaginalis. *Korean J. Parasitol.* **58**, 135–145 (2020).
15. Meri, T., Jokiranta, T. S. & Suhonen, L. Resistance of Trichomonas vaginalis to Metronidazole : Report of the First Three Cases from Finland and Optimization of In Vitro Susceptibility Testing under Various Oxygen Concentrations. **38**, 763–767 (2000).
16. Cudmore, S. L., Delgaty, K. L., Hayward-mcclelland, S. F., Petrin, D. P. & Garber, G. E. Treatment of Infections Caused by Metronidazole-Resistant Trichomonas vaginalis. **17**, 783–793 (2004).
17. Nyirjesy, P., Sobel, J. D., Weitz, M. V., Leaman, D. J. & Gelone, S. P. Difficult-to-Treat Trichomoniasis : Results with Paromomycin Cream. 986–988 (1998).
18. Paulish-miller, T. E. *et al.* Trichomonas vaginalis Metronidazole Resistance Is Associated with Single Nucleotide Polymorphisms in the Nitroreductase Genes ntr4 Tv and ntr6 Tv. **58**, 2938–2943 (2014).
19. Namdari, M., Eatemadi, A., Soleimaninejad, M. & Hammed, A. T. A brief review on the application of nanoparticle enclosed herbal medicine for the treatment of infective endocarditis. *Biomed. Pharmacother.* **87**, 321–331 (2017).
20. Bonifácio, B. V. *et al.* Nanotechnology-based drug delivery systems and herbal medicines : a review. *Int. J. Nanomedicine* **9**, 1–15 (2014).
21. Darani, H. *et al.* Effects of different extracts of Eucalyptus camaldulensis on Trichomonas vaginalis parasite in culture medium. *Adv. Biomed. Res.* **2**, 47 (2013).
22. Al-Ammash, M. S. J. Study the Effect of Alcoholic Extract of Nigella sativa Seeds on Trichomomas vaginalis In Vitro. *Ibn AL- Haitham J. Pure Appl. Sci.* **30**, 10 (2017).
23. Brandelli, C. L. C., Vieira, P. D. B., Macedo, A. J. & Tasca, T. Remarkable anti- trichomonas vaginalis activity of plants traditionally used by the mbyá-guarani indigenous group in Brazil. *Biomed Res. Int.* **2013**, (2013).

24. Al-Snafi, A. E. Traditional uses of Iraqi medicinal plants. *IOSR J. Pharm. www.iosrphr.org* **8**, 32–95 (2018).
25. Marzouk, B. *et al.* Screening of analgesic and anti-inflammatory activities of Citrullus colocynthis from southern Tunisia. *J. Ethnopharmacol.* **128**, 15–19 (2010).
26. Rahimi, R., Amin, G. & Ardekani, M. R. S. A review on citrullus colocynthis schrad.: From traditional iranian medicine to modern phytotherapy. *J. Altern. Complement. Med.* **18**, 551–554 (2012).
27. Giordani, R. B. *et al.* Candimine-induced cell death of the amitochondriate parasite Trichomonas vaginalis. *J. Nat. Prod.* **73**, 2019–2023 (2010).
28. Cox-Georgian, D., Ramadoss, N., Dona, C. & Basu, C. Therapeutic and medicinal uses of terpenes. *Med. Plants From Farm to Pharm.* 333–359 (2019) doi:10.1007/978-3-030-31269-5_15.
29. Panche, A. N., Diwan, A. D. & Chandra, S. R. Flavonoids: An overview. *J. Nutr. Sci.* **5**, (2016).
30. Harsha, N. *et al.* Phytochemical Analysis of Some Selected Spices. *Int. J. Innov. Res. Sci. Eng. Technol. (An ISO* **3297**, 2319–8753 (2007).
31. Tlili, N., Feriani, A., Saadoui, E., Nasri, N. & Khaldi, A. Capparis spinosa leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. *Biomed. Pharmacother.* **87**, 171–179 (2017).
32. Hendra, R., Ahmad, S., Sukari, A., Shukor, M. Y. & Oskoueian, E. Flavonoid analyses and antimicrobial activity of various parts of Phaleria macrocarpa (Scheff.) Boerl fruit. *Int. J. Mol. Sci.* **12**, 3422–3431 (2011).
33. Asli, E. Quantitative analysis of quercetin in different parts of Capparis spinosa by HPLC. **3**, 5775–5778 (2012).

Tables

Table 1
Effect of nano *C. colocynthis* (100, 250 and 500 ppm) on the *T. vaginalis* after 12, 24 and 72 h compared with Metronidazole and negative control.

	Conc. Ppm	12 h	GI(%)	24h	GI(%)	72h	GI(%)	P.value
Nano- Citrullus colocynthis	100	9.4 ± 0.1	17.81 ± 0.884	7 .6 ± 0.513	43.78 ± 3.829	6.13 ± 0.321	53.45 ± 2.491	P ≤ 0.05
	250	8.6 ± 0.208	24.59 ± 1.842	5.83 ± 0.251	57.46 ± 1.878	4.47 ± 0.378	66.37 ± 2.934	P ≤ 0.05
	500	6.47 ± 0.251	43.77 ± 2.227	4.27 ± 0.152	69.15 ± 1.139	1.43 ± 0.351	89.89 ± 2.722	P ≤ 0.05
Metro-Nidazole	500 µg/cm^3	6.5 ± 0.2	43.47 ± 1.769	4.1 ± 0.2	70.40± 1.492	1.8 ± 0.264	87.04± 2.05	P ≤ 0.05
Negatine Control		11.53± 0.493		13.13± 0.251		13.77± 0.808		P ≤ 0.05

*Parasites account x 10^4

Table 2
Effect of nano C. *spinose* (100, 250 and 500 ppm) on the *T. vaginalis* after 12, 24 and 72 h compared with Metronidazole and negative control.

	Conc. ppm	12 h	GI(%)	24h	GI(%)	72h	GI(%)	P.value
Nano- Capparis spinosa	100	9.5 ± 0.1	16.92 ± 0.884	7.83 ± 0.568	42.54 ± 4.243	6.23 ± 0.321	52.67± 2.491	P ≤ 0.05
	250	8.77 ± 0.152	23.41 ± 1.351	6.17 ± 0.115	54.98 ± 0.861	4.53 ± 0.152	65.85± 1.184	P ≤ 0.05
	500	6.53 ± 0.251	43.18 ± 2.227	4.5 ± 0.1	67.41 ± 0.746	1.8± 0.2	87.04± 1.55	P ≤ 0.05
Metro-Nidazole	500 µg/cm^3	6.5 ± 0.2	43.47 ± 1.769	4.1 ± 0.2	70.40 ± 1.492	1.8 ± 0.264	87.04± 2.05	P ≤ 0.05
Negatine Control		11.53 ± 0.493		13.13 ± 0.251		13.77 ± 0.808		P ≤ 0.05
*Parasites account x 10^4								

Table 3
inhibitory concentrations (IC50) against *Trichomonas vaginalis* .

Treatment	IC50	Concentration (PPm)
Citrullus colocynthis	99.001	100
Capparis spinosa	99.495	100–250

Figures

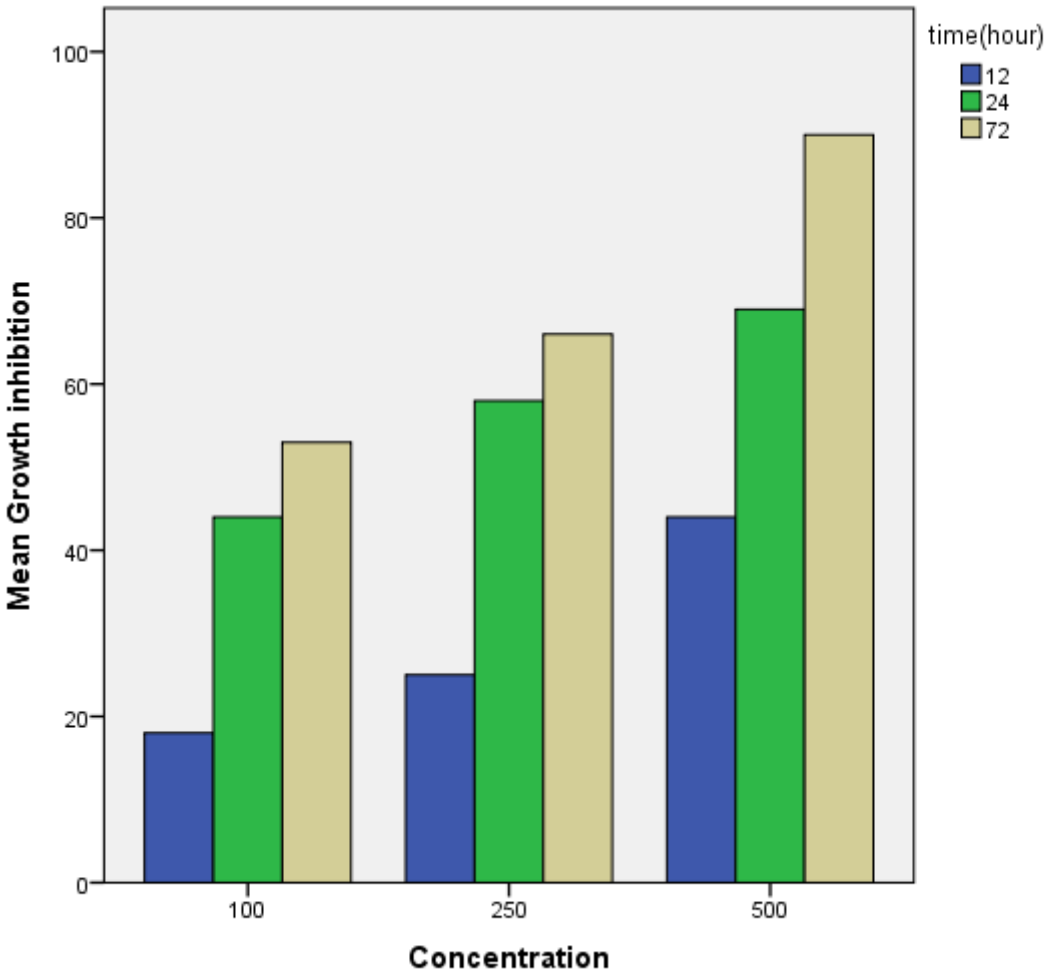

Figure 1

Growth inhibition by Citrullus colocynthis according to the concentration and time Parameters

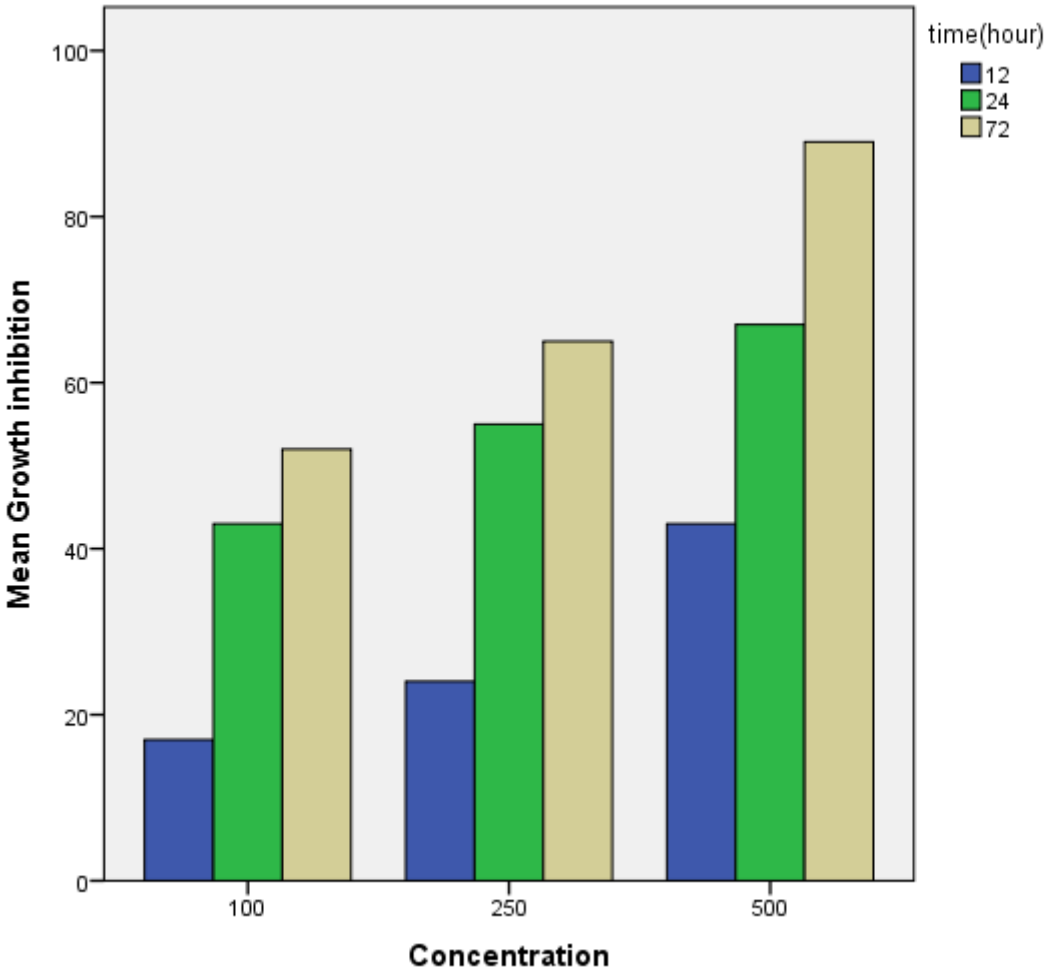

Figure 2

Growth inhibition by Capparis spinose according to the concentration and time Parameters

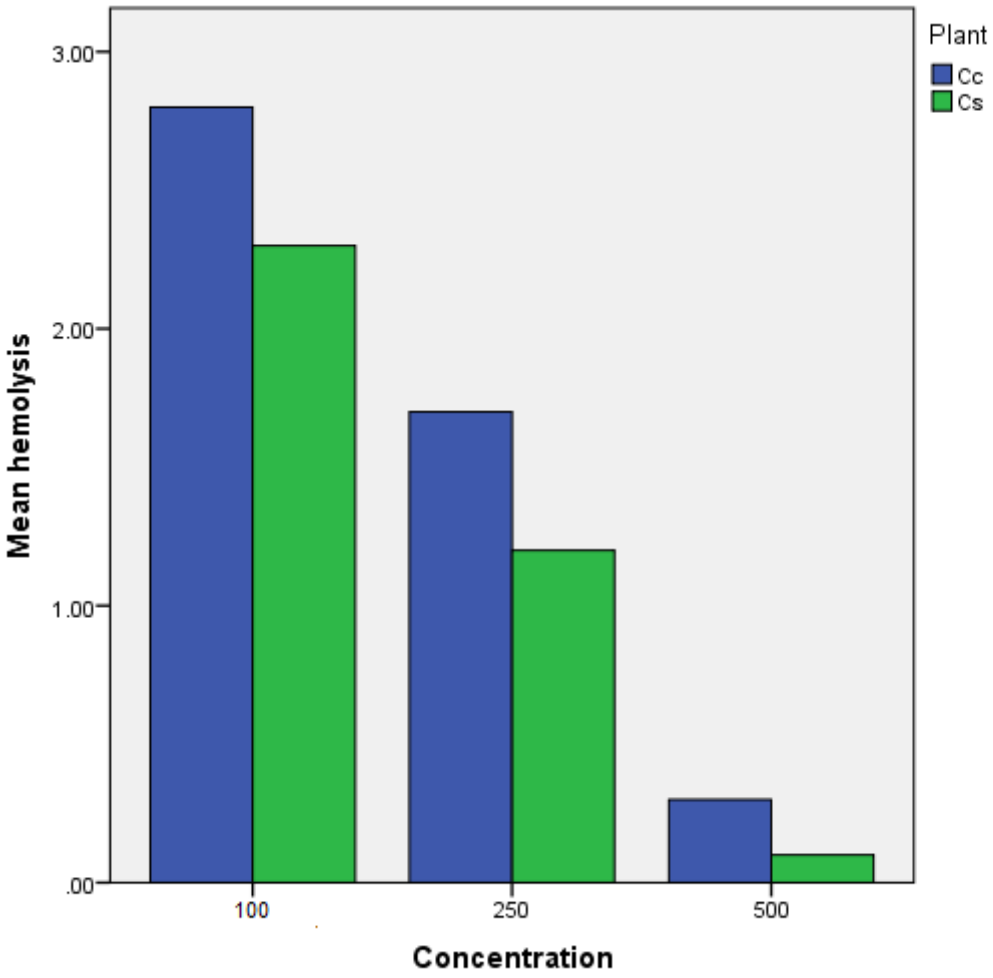

Figure 3

Hemolytic cytotoxicity of Capparis spinose (Cs) and Citrullus colocynthis (Cc)