Original Article

Therapeutic Activity of Solanum Tuberosum L. Extract Against Growth Trichomonas Vaginalis, in Vitro.

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Abstract

Aqueous extract of *Solanum tuberosuml* leaf extract was found as growth inhibitor of trophozoites, *Trichomonas vaginalis* developed in Diamonds' medium (TYM) supplemented with inhibitory human blood plasma at a ratio of 100%, at the concentrations 3, 3.5, 4, 4.5, 5and 5.5 mg. l^{-1} which showed a decrease in the number of cues and in an inverse relationship with the concentrations during the growth periods 24, 48, and 72 hours. The concentration 5.5 mg. l^{-1} caused 78.1% folliculogeni city during 72 hours of growth. It was also noted that this extract had an effect on the number of generations, which decreased when using high concentration of potato leaf extract between 0.25 ± 0.1 to 1.51 ± 0.16 generations in different growth periods compared with control group. 1.79 ± 0.11 to 3.20± 0.07. The LC50 of potato leaf extract was determined as 5 mg. l^{-1} at a time of 72 hours of growth. The treatment with plant extracts is a new field in antimicrobial medicine, and is always an urgent and successive need in the field of Medicine. field for

Keywords: Trichomonas vaginalis; extract; Solanum tuberosum L.

INTRODUCTION

Trichomoniasis (trich) is a highly pathogenic parasitic disease, approximately 276.4 million peoples annually had been infected worldwide, including 3.7 million in the United States suffering from) Trichomoniasis - CDC 2018, Rowley *et al*., 2019) [1,2] transmitted through intercourse (sexual intercourse) between men and women and women together (Lesbianism) WSW and WSWM. (Centers for Disease Control and Prevention 2021, Meites, 2019) [3,4]. *Trichomonas vaginalis*, is an anaerobic protozoan parasite which belongs to the Trichomondidae family. In America, the incidence of trich increased annually by an estimated 3-5 million people(2021) [5]. In men, it is less frequent than women by about 4-5 times, and thus it is dominant in women, Flagg, (2019) [6]. Leading to the emergence of symptoms of the disease, the incubation period of 4-28 days) Coleman *et al.,* 2013) [7]. In women, burning, itching or painful red genitals (vagina, vulva, labia) appear with new, unusual yellow or green vaginal secretions with a foul smell (similar to the smell of fishy odor). Schweb and, (2004) [8]. In men, it is accompanied by itching and irritation inside the penis, then burning after ejaculation or urination, inflammation of the epididymis, prostate and seminal vesicles (cotch *et al*., 1997) [9]. This disease may remain for months or years, depending on its severity. Centers, (2021) [10].

The infection of this disease is sometimes asymptomatic, Daugherty, (2019) [11] negatively affecting the reproductive health of women, including premature birth and infertility SOLOSEC (2021) [12] and premature rupture of the fetal membranes or chorioamnionitis Centers, (2021) [10]. This increases the chance of developing pelvic inflammatory disease (PID) (Wiringa *et al*., 2020) [13] and paves the way for an increase in HIV infection in people (Van, 2016; Masha, 2019) [14,15]. In addition to doubling the joint infections between Trichomoniasis and Acquired Immunodeficiency Syndrome (Kharsany, 2020; price, 2018) [16, 17].

Numerous techniques were used to identify this condition, including the clinical use of a genuine vaginalis speculum by Lossick (1998) [18]. Direct microscopy, culturing (Sood et al., 2007) [19], immunofluorescence, and agglutination of latex particles are examples of other laboratory experiments. (2008) Al-Swehli [20]. Test Jeanne, (2004) [21]; and PCR. Currently, only 5'-nitroimidazole derivatives, such as metronidazole and tinidazole, are used to treat trichomoniasis. The two medications that have been authorized by the Food and Drug Administration for the treatment of T.vaginalis are metronidazole and tinidazole, according to Masha, (2017) [22]. (2017) Bouchemal [23] Nitroimidazoil that penetrates the parasite's trophozoite through passive diffusion Ghosh, (2018) [24]. The cure rate for these suggested medications is almost 95% (Kissinger, 2015; Alessio, 2019) [25,26]. Mabaso (2020) [27] found that 95% of the isolates were resistant to treatment with metronidazole in a recent investigation of pregnant women in South Africa. The US Food and Drug Administration has currently licensed SOLOSEC® (secnidazole) for the treatment of trichomoniasis; after therapy, the cure rate ranged from 91.7% to 100% over the course of 2–20 days (2021) [12]. In order to evaluate the impact of plants on the variables producing vaginal infections, many attempts were conducted. In recent investigations, new medications

against trichomoniasis have been presented. In addition to having few adverse effects, herbal medicines have been tried and are thought to be safe and efficient. the fact that it is renewable [28] Ertabakla, 2009

MATERIALS AND WORKING METHODS.

The source of the parasite used in the study.

The parasite *T. vaginalis* was obtained from female patients who attend private medical clinics for obstetricians and gynecologists and from Al-Batool Teaching Hospital in Nineveh Governorate.

Collection of vaginal swabs.

Samples were taken from women who suffer from signs of illness such as burning, itching, and vaginitis (Borchardt *et al*., 1992; Bafghi *et al*., 2009) [29,30] and vaginal secretions, which have an unpleasant odor, Al-Swehli (2008) [20], using a probe sterile speculum, these swabs were taken from the back vault of the cervix, posterier fonrix of cervix, and the walls of the vagina by a sterile cotton swab, which rotated before it was withdrawn, Gwendolyn (1996) [31], and with the help of a specialist, and then dipped in sterile test tubes containing 2 cm^3 of physiological solution (Thomas *et al*., 1996) [32].

Parasite growth in culture media.

The parasites were grown in Diamond's Medium Tryptone, Yeast, Maltose (TYM) (Diamond, 1957) [33] culture medium, inoculated by adding 0.1$cm^3$of positive culture of *T .vaginalis*, uncontaminated, at the age of 3 days, i.e. within the logarithmic phase of growth in glass bottles. Contained on 4.9 cm^3 of the new culture medium with an initial number of 1 x 10^5 cells/cm^3 and this was done under sterile conditions, then the bottles were incubated at 37 °C and the cultivation process was repeated every 3 days.

Parasite count

The number of *T .vaginalis* was calculated in the culture every 24, 48, 72 hours using a heamocytometer slide of the type Neubaeur, 0.9 cm^3 was taken from the culture in which the number of parasites is to be calculated and 0.1 cm^3 of 40% formalin solution was added to it. It is equivalent to one drop for the purpose of fixing the parasite during counting. The enumeration process was carried out using a light microscope lens under a magnification of 100x and 400x.

Plant material:

Potato, *Solanum tuberosum* L. belongs to solonace (Nightshades) family (Majaz *et al*., 2018) [34] It has upright stems and branches that bulge under the soil in the form of tubers in which nutrients are stored. The leaves are compound oval, and these leaves are poisonous. AL-Rubaiay (2013) [35] It used to treat worms, burns, tumors under the eye, insect bites and resist cancerous growth.

Active ingredients

A source of a steroidal ring glycosidic alkaloid, known as Solanine $C_{45}H_{73}NO_{15}$, which is found in green potato tubers and leaves. Atlas Plant 2012 [36]. Acrylamide, which would have properties that lead to toxicity to the nervous system, and negative impact on the health of the reproductive system, (Semla *et al*., 2017 [37]. It is a rich source of phenolic compounds and vitamins such as quercetin, which has an effective role in reducing inflammation.

Preparation of the aqueous extract

The aqueous extract was prepared according to the method (Riose *et al*., 1987) [38] and the concentrations 3, 3.5, 4, 4.5, 5, and 5.5 mg l^{-1} were applied culture medium to determine the extent of its effect on the growth of generations and the number of *T.vaginalis* parasites compared with the control group according to the method (Benjamin and German., 1993) [39].

Statistical analysis:

Complete random design (CDR) was used in the analysis of the trials and it was statistically tested by using Bruning, Duncan multiple range test Bruning, (1977) [40] in all trials, to see if there is a significant difference between the treated and untreated (control) indices with the extracts. Water according to the concentrations used at the level of significance $p \leq 0.05$ Revision, writing and modification.

RESULTS

Concentrations 3, 3.5, 4, 4.5, 5and5.5mg. l^{-1} of raw aqueous extract of potato leaves were used to show its inhibitory effect on the growth of *T.vaginalis* and compared it with the control group as shown in Table (1) which shows a reduction in the preparation of the parasite with an inverse relationship with the increase in the different growth periods, and when analyzing the results statistically, it indicated that there were significant differences at the probability level of $p \leq 0.05$ between the growth rate of *T.vaginalis*, treated with potato leaf extract and the control group, where this extract had a clear inhibitory effect on the growth of the parasite and showed the concentration that caused inhibition of parasite growth 50% of the *T.vaginalis* within 72 hours of growth. LC50 (Lethal Concentration & Time) is 5 mg. l^{-1} The percentage of growth of *T.vaginalis* decreased from 71.9% to 30.8% when this extract was added at concentrations ranging between 3-5.5 mg. l^{-1} within 24 hours of growth. While this percentage decreased from 83% to 17.4% within 48

hours, and after 72 hours, it led to a decrease in the growth rate from 78.3% to 31.4%. Table (2) shows the effect of the raw aqueous extract of potato leaves on the number of generations of indicators, which led to a decrease in the number of generations in an inverse relationship with the increase of these concentrations. The results of the statistical analysis showed that there were significant differences at the probability level $p \leq 0.05$ between the rates of the number of generations for the cues treated with potato leaf extract and the control group during the different growth periods. The number of generations ranged from 1.36 ± 0.087 generations to 0.25 ± 0.1 when using concentrations from 3 - 5.5 mg.l^{-1} Compared to the control group 1.79 ± 0.11 generations within 24 hours of growth, the number of generations ranged between 2.27 ± 0.11 to 0.56 ± 0.20 generations, compared to the control 2.28 ± 0.9 generations within 48 hours, while at the time 72 hours, the number of generations ranged between 2.76 ± 0.07 to 1.51 ± 0.16 GEL compared to the control group 3.20 ± 0.07 GEL.

DISCUSSION

The result of this study showed that the growth of *T. vaginalis* was inhibited by the aqueous extract of potato leaves, which was similar to the result of (Al-Sultan *et al*., 2005) [41] who used the aqueous extract of *Trigonell foenum graecuma* only and a mixture of *T. f. graecum* and *Matricaria chamomilla*, *T .f. graecum* and *Thymus vulgaris* at a concentration of 10 mg.l^{-1}, which led to 100% inhibition of parasite growth at times 24,48, 72,hours thus the superiority of fenugreek plant over the water extract of potato leaves at the time, while the aqueous extract of potato leaves outperformed it in concentration. While this percentage decreased from 83% to 17.4% within 48 hours, and this was the result of the aqueous extract of the leaves of the. The reason for inhibiting the growth of the *T. vaginalis* to 88% within 48 hours with the result (Hosseini *et al*., 2013) [42]. who used the aqueous extract of *Eucalyptus Camaldulensis* at a concentration of 12.5 mg. l^{-1}, which caused the inhibition of the growth of *T. vaginalis* to 80% within 48 hours, with the potato plant superior to *E. Camaldulensis* in the concentration that exceeded it at the same time. After 72 hours, the growth rate decreased from 78.3% to 31.4%, and the result of the current study differed from the result of (Sereshti *et al*., 2012) [43], who used the aqueous extract of *Stachys sylvatica*, and after 72 hours in the medium, which had no effect on *T. vaginalis*, However this result is converged with the result of (Chalechale *et al*., 2019) [44] who used a mixture of volatile oils extract of *Coriandrum sativum* leaves and Metronidazole at concentrations 0.250 and 0.5 mg. l^{-1}, which led to the killing of all *T. vaginalis* within 90 minutes. It was close to the result of (Khalili *et al.,* 2011) [45]. who used aqueous extract of *Juglans regia* at concentrations of 800, 400 and 800 mg. l^{-1}, respectively, which led to the destruction of all parasites. Potato leaf extract was superior to in it concentration

It is comparable to the findings of (Zarea et al, 2013) [46], who employed an aqueous extract from the Taxus baccata plant to limit the growth of T. vaginalis in culture conditions. At doses of 0.2, 0.3, 0.4, and 0.5 mg l-1, the 60% component of crude extract indicated greater than 90% (p 0.05). 60% extract at a dosage of 0.2 mg l-1 caused 100% inhibition, however 90% of the component indicated 60% growth inhibition. As a result, this outcome surpassed the potato leaf extract's ability to concentrate when it employed T. baccata plant's aqueous extract.

The inhibitory effect of the aqueous extract of the leaves of the potato plant may be attributed to the fact that it contains some chemical compounds, the most important of which are alkaloids, glycosides and phenols such as solanine and quercetin.

CONCLUSION

The aqueous extract of potato leaves had a clear effect on the vitality of the trophozoite, which led to its reduction and destruction, which was inversely proportional to the increase in concentration and exposure time as shown in Table (1). Thus, it was suggested that a future study be conducted to isolate the effective compounds of potato leaves and may the extent of their impact on the vitality of the trophozoite of *T. vaginalis* parasite *in vitro* and *in vivo*.

Table No. 1 Effect of aqueous extract of potato leaves on the number of *T. vaginalis* 10^5×*T.vaginalis* at different time periods (the number of *T. vaginalis* used in culture is $1×10^5$cells/cm^3).

incubation period hour / transaction Conc. mg.ml^{-1}	24. hour		48. hour		72. hour	
	Average* ± standard deviation	% **growth**	**Average* ± standard deviation**	% growth	**Average* ± standard deviation**	% growth
Control	3.60 ± 0.3 f	100	6.2 6± 0.3 d	100	9.1±0.3 a	100
3 mg.ml^{-1}	2.57 ± 0.2 ghi	71.9	3.20 ± 0.4 fg	83	7.13 ±0.2 b	78.3
3.5 mg.ml^{-1}	2.35 ± 0.1 hij	65.2	3.20 ± 0.3 fg	51.1	6.63±0.4 c	73.6
4 mg.ml^{-1}	1.90 ± 0.1 hij	52.8	2.80 ± 0.3 gh	4.47	5.37±0.6 d	59
4.5 mg.ml^{-1}	1.67 ± 0.4 klm	46.4	2.0 ± 0.4 ijk	31.9	5.08±0.4 de	56.4
5 mg.ml^{-1}	1.16 ± 0.1 lm	32.2	1.86 ± 0.3 jkl	29.7	4.56±0.4 e	50.1
5.5 mg.ml^{-1}	1.11 ± 0.1 m	30.8	1.09 ± 0.2 lm	17.4	2.86 ± 0.5 gh	31.4

*The number represents the average of three replicates ± standard deviation.
*The different letters indicate the presence of moral differences, while the similar letters indicate the absence of significant differences according to the Duncan test at the level of morale. ($p \leq 0.05$).

Table No. 2 Effect of aqueous extract of potato leaves on the number of generations of *T. vaginalis* in different growth periods (the number of *T. vaginalis* used in culture is 1×10^5cells/cm^3)			
incubation period hour / **transaction** Conc. mg.ml^{-1}	**number of generations Average* ± standard deviation**		
	24. hour	**48. hour**	**72. hour**
Control	1.79 ±0.11 ef	2.2 8± 0.9 d	3.20 ± 0.07 a
3 mg.ml^{-1}	1.36 ±0.087 ghi	2.27 ± 0.11 d	2.76 ± 0.07 b
3.5 mg.ml^{-1}	1.75 ±0.09 hij	1.70 ± 0.13 efg	2.68 ± 0.04 bc
4 mg.ml^{-1}	0. 9 ±0.3 jk	1.52 ± 0.12 fgh	2.41 ± 0.14 cd
4.5 mg.ml^{-1}	0.71 ±0.4 lm	1.07 ± 0.30 jk	5.08 ±0.4 de
5 mg.ml^{-1}	0.36 ±0.1 nop	0.86 ± 0.25 kl	2.19 ± 0.10 d
5.5 mg.ml^{-1}	0. 25 ± 0.1 op	0.56 ± 0.20 mn	1.51 ± 0.16 fgh

*The number represents the average of three replicates ± standard deviation
*The different letters indicate the presence of moral differences, while the similar letters indicate the absence of significant differences according to the Duncan test at the level of morale. ($p \leq 0.05$).

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