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Comparison of the Effect of Metronidazole, Tinidazole, Mango and Blueberry Extracts on *Trichomonas vaginalis* in Vitro

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Background: Trichomoniasis is the most common nonviral sexually transmitted human disease that is caused by protozoan *Trichomonas vaginalis*. Metronidazole is the selective drug in trichomoniasis treatment. However, the reported cases show an increasing trend of drug resistance. This study aimed to evaluate the effect of mango and blueberry extracts on *T. vaginalis*.
Materials and Methods: *T. vaginalis* was cultured axenically in TYM (Trypticase Yeast Extract) medium supplemented with 10% bovine serum. The effect of mango and blueberry extracts at 50, 100, 200, 400, 800 and 1000 µg.mL^{-1} on *T. vaginalis* was studied after 24 and 48 hours. The final numbers of parasite with a hemocytometer and Trypan blue were recorded. Then the value of IC_{50} [Half maximal inhibitory concentration] and the lethal percent were calculated. In the present study, the metronidazole was used as positive control. The IC_{50} value of metronidazole and tinidazole were calculated in the concentrations of 0.02, 0.04, 0.08, 0.16 and 0.32 µg.mL^{-1}.
Results: The final results confirmed the significant effect of all mango and blueberry extracts concentrations on the reduction of parasite numbers (P-value<0.05). The extract concentrations of 1000 µg.mL^{-1} had the most significant effect on *T. vaginalis* growth inhibition after 24 hours. The IC_{50} values of mango and blueberry extracts, metronidazole, and tinidazole were calculated as 118.3, 60.74, 0.042 and 0.02 µg.ml^{-1} respectively.
Conclusion: Based on the obtained results, the different concentrations of mango and blueberry extracts have significant anti *Trichomonas vaginalis* activities. It is suggested carrying out further studies on suitable animal models.

Keywords: Trichomonas vaginalis, Mangifera, Blueberry plant, Metronidazole, Tinidazole, Inhibitory concentration 50

1. Background

Chemotherapy of many parasitic infections remains an unsolved problem. Extensive resistance to drug is emerging, not only among helminthic diseases (1) but also in protozoan infections such as *Trichomonas vaginalis*, the most general parasite causing sexually transmitted diseases (2). *T. vaginalis* is a mucosal pathogen that affects the human urogenital tract causing trichomonosis (3). According to WHO, more than 170 million people are infected with *T. vaginalis* annually worldwide (4). Its typical symptoms are vaginitis and cervicitis in women; in men the infection can lead to chronic prostatitis syndrome (5). Trichomoniasis increases the risk of human immunodeficiency virus infection (6) and is associated with miscarriages (6) and cancer of the cervix (7). The most important clinical signs of trichomoniasis are vaginal or urethral discharge, foul-smelling discharge, pruritus, severe irritation, dysuria, edema or erythema, and abdominal pain. In pregnant women, trichomonads are implicated in premature membrane rupture, premature labor, and delivery of low birth-weight babies (8). Metronidazole and tinidazole are the two drugs of choice used for the treatment of human trichomoniasis. However, potential carcinogenic, teratogenic, embryogenic effects and clinical and laboratory-generated drug-resistant isolates of *T. vaginalis* have been reported(9). General adverse reactions include urticaria, glossitis, headache, vertigo, nausea, pruritus, vomiting, bitter metallic taste, dry mouth, and a disulfiram-like reaction with ingestion of alcohol. More serious side effects are uncommon but include leukopenia, palpitation, confusion, eosinophilia, and various central nervous system effects (10). In order to improve the current chemotherapy of *T. vaginalis* infection, medicinal plants could be a source of new antiprotozoal drugs with high activity, low toxicity, and a lower price.

2. Objectives

The aim of this study was to estimate the in vitro trichomonacidal activity of the ethanolic extracts of mango and blueberry. As far as our literature study could establish, no report is available for the trichomonacidal activities of these plant species in the literature.

3. Materials and methods

3.1. Preparation of crude extracts

Preparation of the ethanolic extract for screening of antitrichomonal activity was carried out with 20 g of powdered plants material that was macerated in 300 mL of ethanol for 1 week at room temperature. After filtration, the solvent was then removed by rotary evaporator. The concentrate was dried by vacuum freeze dryer to obtain a dry extract and stored at −20 °C for further analysis in tightly sealed glass vials (11).

3.2. Parasites

Trophozoites of *T. vaginalis* were maintained in TYM (trypticase yeast extract) medium supplemented with 10% bovine serum. The trophozoites were axenically maintained, and for the assays, were employed in the log phase of growth.

3.3. In vitro susceptibility assays

The 10^4 CFU.mL^{-1} of *T. vaginalis* trophozoites were incubated for 24 and 48 hr at 37 °C in the presence of

different concentrations (50–1000 µg.mL^{-1}) of the crude extracts and metronidazole and tinidazole (0.02, 0.04, 0.08, 0.16, 0.32 µg.mL^{-1}) in dimethyl sulfoxide (DMSO) separately. Each test included metronidazole as positive control, a control (culture medium plus trophozoites and DMSO), and a blank (culture medium). After that the final number of parasites was determined with a hemocytometer and vitaldyetrypan blue. The results were obtained from at least three independent experiments, in triplicate, and expressed as the percentage of living parasites after 24 and 48 hr of incubation considering motility and normal morphology (percentage of living organisms compared to negative control). The concentration required to inhibit 50% of the parasites growth (IC_{50}), was determined by nonlinear regression (2). The IC_{50} was calculated by probit analysis (GraphPad Prim5 software) (12).

3.3. Cytotoxicity against the VERO cell line

Cytotoxicity of host cells is a very important decisive factor for assessing the selectivity of observed antitrichomonal activity. 10^4 viable cells from the cell line were seeded in a 96-well plate (Costar) and incubated for 24 hr. Vero (African green monkey kidney) cells were grown in DMEM (Gibco) media supplemented with 10% (vw^{-1}) fetal bovine serum (FBS; Gibco) with 100 UmL^{-1} penicillin and 100 µg.mL^{-1} streptomycin and maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. When cells reached >80% confluence, the medium was replaced, and the cells were treated with the mango and blueberry extracts in 50, 100, 200, 400, and 800 µg.mL^{-1}. Amounts of 0.02, 0.04, 0.08, 0.16 and 0.32 µg.mL^{-1} of metronidazole and tinidazole, dissolved in dimethyl sulfoxide (DMSO) at a maximum concentration of 0.05%, was added to each well and incubated at 37 °C for 4 hr. DMSO was added and the plates incubated for 15 min to stop the reaction and to dissolve the insoluble purple formazan. The amount of MTT-formazan present is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 550 nm. Three wells per dose were analyzed in three diverse experiments, and the results were expressed as the percentage of viable cells in comparison to the negative control (untreated cells). Metronidazole was used as a positive control, while untreated cells were used as negative controls. The concentration of the crude extract that killed 50% of the cells (CC_{50}) was calculated by GraphPad Prim 5 software. The selectivity index (SI) of the extracts, defined as the ratio of cytotoxicity of biological activity (SI=CC_{50} vero cells/IC_{50} *T. vaginalis*), was then calculated. The data were subjected to one-way analysis of variance (ANOVA) using a possibility value of P<0.05. Tukey's test was used to identify significant differences between means among the different treatments (GraphPad Prim 5 software) (12).

4. Results

The results for antitrichomonal activity are shown in tables1 and 2 and figures 1, 2 and 3. Moderate activity was observed with the mango and blueberry extracts. Mango and blueberry extracts showed cytotoxic effects against the *T. vaginalis* tested. Blueberry extract showed higher cytotoxicity than mango. The blueberry presented the highest anti *Trichomonas vaginalis* activity than mango at all concentrations tested.

Table 1. Anti-Trichomonal activity (IC_{50}), cytotoxic activity on vero cells (CC_{50}) and selective index (SI) of mango and blueberry extracts, Metronidazole, Tinidazole (mean ±SD:n+3).

Sample	IC_{50} (µg.mL^{-1})	CC_{50} (µg.mL^{-1})	SI
Metronidazole	0.047	70.23	1494.25
Tinidazole	0.02	73.27	3650
Mango	118.3	58060	490.78
Bluberry	60.74	37319	612.79

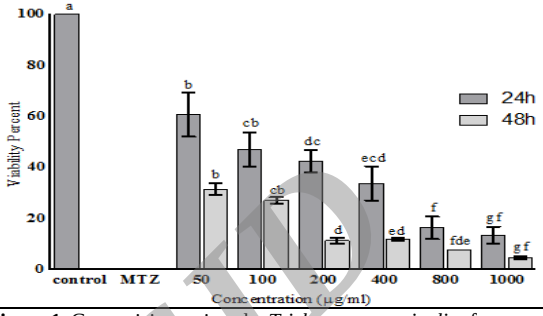

Figure 1. Cytotoxicity against the *Trichomonas vaginalis* after treatment with mango extract at different concentrations. Data represent means± standard deviation of at least three experiments. Different letters (a, b, c, d) at the same concentration indicate significant differences among the compounds (P-value <0.05).

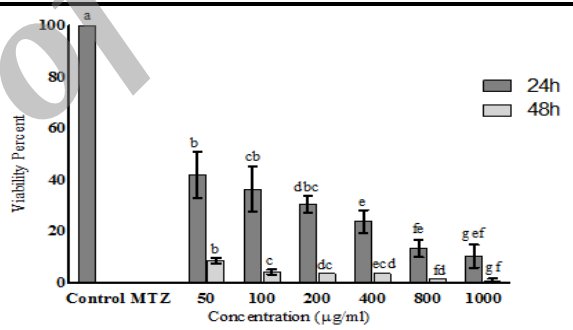

Figure 2. Cytotoxicity against the *Trichomonas vaginalis* after treatment with blueberry extract at different concentrations. Data represent means±standard deviation of at least three experiments. Different letters (a, b, c, d) at the same concentration indicate significant differences among the compounds (P-value <0.05).

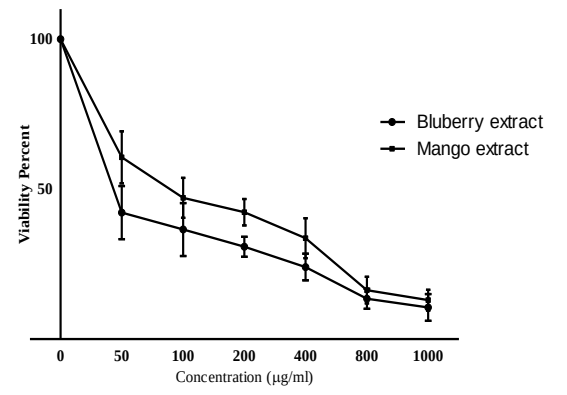

Figure 3. Cytotoxicity of mango and blueberry extracts against the *Trichomonas vaginalis* in 24hr. Data represents mean±SD of at least three experiments.

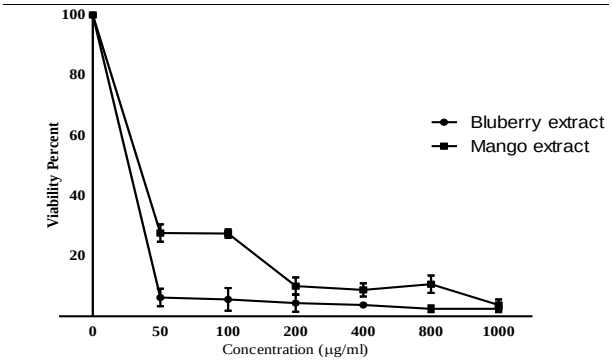

Figure 4. Cytotoxicity of mango and blueberry extracts against the trichomonas vaginalis in 48hr. Data represent mean±SD of at least three experiments.

Discussion

The standard dose of metronidazole resistance in *T. vaginalis* is rising, and due to the side effects of metronidazole, related research is in progress to find an effective treatment for *T. vaginalis* (12). Thus, numerous important studies on herbal remedies are being carried out that include fewer side effects on the body, cheapness and availability, adverse effects of drugs such as metronidazole adverse effects on children and pregnant women. Mango is a tropical fruit in many areas of the world and also among the products that are cultivated in southern Iran. Gas chromatography has previously shown that the ethanolic extract of mango kernel contains several phenolic and alkaloids compounds (13). Flavonoids and other phenolic compounds in plants are widely spread. Their various properties including antioxidant, antibacterial, and ant-iinflammatory effects have been reported in many studies (14).

Studies have shown that the mango kernel also contains saponins, flavonoids, glycosides, tannins, and alkaloids (15), while the alkaloids have antiparasitic properties. Alkaloids have anticancer, antihelminthicand antiparasitic properties. The study conducted in 2010 by Jasminer and colleagues, have shown that the mango kernel has antibacterial effects and showed that MIC values of mango extract is less than 4.7 micrograms per milliliter (15). In a study carried out in 1994 by Ponce and colleagues, mango extract was shown to have significant antiprotozoan parasite *Giardia lamblia* activity (16). In a study done by Sunday et al. in 2005, antitrypanosoma activity of mango tree root extract was reported (17).

Blueberries contain large amounts of phenolic compounds such as flavonoids, tannins, and anthocyanins (18). Anthony et al. at 2007 studied the effect of blueberry extract on *Giardia lamblia* and showed thepolyphenolic and anthocyanin compounds as active ingredients that kill the parasite trophozoite (19).

In this study, the polyphenolic compounds had lethal effect on parasite. In our study, after treatment of parasites with mango and blueberry extracts, it was observed that with increasing concentration and time, there was reduction in survival and reproduction of the parasite. Since this study did not determine active compounds in extracts, based on the studies by other researchers on mango kernel extracts, it is likely that antitrichomonas activity of mango kernel extract is due to the polyphenolic compounds and alkaloids found in it.

In this study, the effect of blueberry extract on Trichomonas was much stronger than the mango kernel extract; this is perhaps due to that the amount of phenolic compounds presented in it, is higher than mango.

Conflict of Interests

Authors declare they have no conflict of interests.

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Authors' Contribution

All authors contribute in writing different parts of this manuscript.

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