**In Vitro Effect of Pomegranate Extract on Trichomonas Tenax**

**Gehad T. El-Sherbini***
Department of Parasitology, Faculty of Pharmacy, University Cairo, Egypt

---

**Abstract**

The incidence of *T. tenax* in patients with acute ulcerative gingivitis has been demonstrated in several published reports about it. Metronidazole was known as the most effective drug for human trichomoniases, however, drug resistance and toxicity appeared. This study was designed to investigate the in vitro inhibitory activity of *P. granatum* ethanol extract on the growth and motility of *Trichomonas tenax* in comparison to metronidazole. Pomegranate ethanol extract group was divided into four groups with concentration of 12.5, 25, 50, and 100 mg/ml, respectively, metronidazole group and blank control. Metronidazole group was given 10 μg/ml of the drug. Each group had 4 wells with 125 μl *T. tenax* (2×10⁵/ml). At 12 hr, 24 hr, 48 hr and 72 hr after drug treatment, the anti- *T. tenax* effect of pomegranate ethanol extract was tested by microscope counting method. The results showed that the better effect on anti-*T. tenax* 60% pomegranate ethanol extract group with concentrations of 12.5 mg/ml and 25 mg/ml showed higher anti- *T. tenax* (P<0.01). The ethanol extract of pomegranate granules has a remarkable effect on *T. tenax*, and among the groups, 60% ethanol extract shows the best anti-*T. tenax* activity.

**Keywords:** Trichomonas tenax; Punica granatum; Metronidazole; Herbal medication

---

**Introduction**

The human oral cavity is home to numerous microorganisms. *Trichomonas tenax* (*Trichomonas buccalis*) is a regular guest of human oral cavity microorganism [1]. It is an anaerobic species that lives as a commensal in the mouth of human oral cavity. It is frequently associated with pyogenic organisms in pus pockets or at the base of teeth. There are studies that relate to its prevalence in patients with Marginal Chronic Periodontitis [2]. Transmission is through saliva, droplet spray, and kissing or use of contaminated dishes and drinking water [3]. World widely, its prevalence in the mouth ranges from 4 to 53% [4,5].

The detection of *T. tenax* in the human oral cavity is an indication of hygiene poor oral, so that its incidence increases significantly in patients with periodontal problems, this being three to four times higher than in periodontal healthy subjects [6].

Since the organism is believed to enter the respiratory tract by aspiration from the oropharynx and then cause bronchopulmonary trichomoniasis, the importance of oral infections has been increased recently [7].

The development of drug resistance in human pathogens against commonly used treatment has necessitated a search for new therapeutic agents from other sources. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms [8,9], and many compounds of plant products have been shown to be specifically targeted against resistant pathogens [10].

*Punica granatum*, which belongs to the family of Puniceae, is commonly known as pomegranate, grenade, granats and punica apple [10]. *Punica granatum* has been used extensively as a traditional medicine in many countries [9] for the treatment of dysentery, diarrhoea, helmintiasis, acidosis, hemorrhage and respiratory pathologies [11,12]. In addition, *P. granatum* is reported to have antioxidant [9,10] anti-atherosclerotic [13,14], antibacterial [15,16] and antiviral [17] properties. The constituents of *P. granatum* include galloactechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are very well known for their therapeutic properties [18]. *Punica granatum* peel is used to treat infections found in human sexual organs as well as mastitis, acne, folliculitis, pile, allergic dermatitis, tympanitis, scalds, diarrhea, dysentery and as an antioxidant. In addition, it is reported that the extracts of *P. granatum* have antimicrobial activity against *Salmonella* [18].

Pomegranate components have properties that could promote oral health, including reducing the risk of gingivitis. However, to date, no studies regarding the anti-*T. tenax* activity of *P. granatum* extract have been conducted. Therefore, the goal of this study is to evaluate the anti-*T. tenax* activity of the extracts of *P. granatum* peel in vitro.

**Materials and Methods**

**Patients**

The periodontiums of 51 patients were clinically examined, and diagnosis and classification of the periodontium was done according to the Periodontal Screening and Recording (PSR) I, in agreement with the Military Academy of Periodontology and Dental Association criteria. Twenty patients were diagnosed with gingivitis (EG1), 22 with periodontitis (EG2) and 9 presented a healthy periodontium (CG). The patients were also asked about the use of medications and systemic conditions which might predispose them to the development of periodontal disease.

**Sample collection**

Samples of saliva and dental biofilm/calculi were collected from all patients in the morning, before any oral hygiene. After determining the frontal mandibular area most affected by periodontal disease (by means of PSR), dental biofilm/calculi samples were collected by scraping the area with sterile periodontal curettes. Unstimulated saliva sam-

---

*Corresponding author: Gehad T. El-Sherbini, Department of Parasitology, Faculty of Pharmacy, University Cairo, Egypt, E-mail: gody_55@yahoo.com

Received April 09, 2012; Accepted June 08, 2012; Published June 14, 2012

Citation: El-Sherbini GT (2012) In Vitro Effect of Pomegranate Extract on Trichomonas Tenax. J Bacteriol Parasitol 3:143. doi:10.4172/2155-9597.1000143

Copyright: © 2012 El-Sherbini GT. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
ples were collected as recommended by Navazesh. All samples were placed in sterile Petri dishes and diluted with saline at room temperature (25 to 28°C). Immediately after dilution, the samples were examined under a light microscope.

For the removal of dental calculus, as well as debris and plaque subgingival periodontal curettes used tartrectomos previously sterilized and, taking the material with the latter the bottom of the periodontal pockets of patients with PMC were also taken plaque samples tooth around the neck of the teeth of patients, employing previously sterile curettes. Dental calculus removed from the experimental group, was ground prior to planting and microscopic observation, using previously sterilized glass rods.

**Transport of samples**

The samples were placed in vials containing transport medium (sterile Ringer solution) and taken to the laboratory for further planting and microscopic observation.

**Inoculation of the samples**

Once the samples were taken to the laboratory, 0.1 ml was taken. Ringer’s solution containing the inoculum, using sterile 1 ml pipettes, inoculating the above-mentioned amount in the broth selective Kupferberg, used for the growth of *T. tenax* ("Kupferberg Trichomonas Broth." Difco Laboratories, Detroit, Michigan, USA), to which were added 0.1 g of chloramphenicol to prevent the growth of bacteria and other microorganisms. Two plantings were made for each patient, an aerobically and anaerobically another, using the jug designed for this purpose (Gas Pak). Seeded culture media were taken to the oven at 37°C for 72 hours.

**Microscopic observation**

For the identification of *T. tenax* microscopic observations were made three times for each patient in order to determine what opportunities exist in the ability to view the scourge. These observations were made in the first instance on the same day of sampling, taking a drop of inoculum containing transport medium with the previously sterile platinum loop and placed onto a glass slide. The two remaining microscopic observations were performed at 72 hours of selective media incubated in an oven, taking a drop in the previously sterile platinum loop, both the stock that was planted under aerobic conditions as was shown in the anaerobic placing in each case on the surface of its respective blade slide.

In each of the cases described above, was placed on the drop of a slide coverslip microbial suspension, then the observation for the cool, using the light microscope (Leitz), focusing first with a low magnification lens (10 ×) and then with higher magnification lenses (20 × 40 ×).

**Extraction of plant material**

**Preparation of the plant extract:** Firstly fresh pomegranates (500 gram) were obtained (in order to prepare fresh extraction) from a public market. The peels of pomegranate were separated and oven dried at 33°C for 7 days. The dried peels were powdered in an electric grinder and stored in plastic bags for the next step. A 100 gm sample of powder was extracted using 200 ml methanol (99.9%) in an electric blender for 30 min. This suspension was filtered three times per day for 30 days. New methanol dissolvent was used each time. Then methanol was removed in a rotary evaporator to produce a dry powder. The final material was dissolved in methanol for obtaining concentrations of 4 mg/ml, 8 mg/ml and 12 mg/ml of dry plant powder [17-19].

**Metronidazole**

It was supplied as 500 mg tablets (Rhone Poulenc Rorer, France). Tablets were dissolved in distilled water, and then diluted in incubation medium to yield 12.5 μg/ml, 25 μg/ml and 50 μg/ml and 100 μg/ml [20].

**Growth inhibition assay**

The effect of *P. granatum* on the growth of the *T. tenax* trophozoites was studied as follows: 2x105 trophozoites were incubated in Selective Kupferberg (KTB) medium with drugs in different concentrations: *p.*
granatum and metronidazole (12.5 µg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml), for 12, 24, 48, and 72 h at 37°C. In addition, controls were included (cultures containing only the parasites) and submitted to the same procedure used for the experimental cultures.

Evaluation of the drug efficacy was done by:

1. Counting the number of trophozoites using the haemocytometer (Neubauer cell-counter chamber).
2. Calculation of the percent of inhibition of multiplication according to the equation:
   \[ \text{Percent inhibition of growth} = \frac{a - b}{a} \times 100 \]
   Where:
   - \( a \) = Mean number of trophozoites in control tubes and
   - \( b \) = Mean number of trophozoites in test tubes [21].
3. Calculation of the percent of motility of trophozoites which is the ratio of motile to total number of parasites counted per 10 High Power Field (HPF).
4. The Minimal Lethal Concentration (MLC) of \( P. \) granatum extract and metronidazole was determined.

Results

The observation in the cool of the sheets prepared allowed us to visualize where they were considered positive, the flagellate in some cases moving in a circular motion and other times not moving and isolated. Usually, the flagellar morphotypes of \( T. \) tenax can be seen with four flagella, but sometimes can be seen only with one or two flagella (Figures 1-4).

The present study was carried out to investigate the \textit{in vitro} activity of \textit{Punica granatum} peel (EEPGP) on the growth and motility of \( T. \) tenax, compared to the standard drug metronidazole. The results showed that the degree of growth inhibition was dependent upon the concentration of \( P. \) granatum and metronidazole.

Effect of \( P. \) granatum extracts in specified times and concentrations on \textit{Trichomonas tenax} were assessed. The inhibitory effect of extract on \textit{Trichomonas} was assessed by counting the live parasites 1, 2 and 24 hours after exposure with extracts.

Findings of this study showed that ethanol extract \( P. \) granatum at concentration of 12.5, and 25 mg/ml killed 80% and 70% after 24 hrs and 60% and 20% of the parasites after 24 hour of exposure Moreover killed 98% of parasites at concentration of 12.5 mg/ml after 12 hours of exposure (Table 1).

Lower dose of metronidazole (12.5 µg/ml) showed complete inhibition of growth after 72 hours (Table 2).

Both of \( P. \) granatum and metronidazole were able to inhibit the motility of the parasite with increasing percent of immotile trophozoites after 72 hr.

Discussion

In the recent years, the use of plants with preventive and therapeutic effects contributes to health care needs [22].

There are three main reasons to be interested in the treating and healing power of plant extract. First, pharmacological studies have demonstrated that many of plants are known to possess antimicrobial agents; second, people are becoming aware of the side effects associated with the over prescription of traditional antibiotics; third, time to time resistant microorganisms against antibiotics are increasing Among these plants, \textit{punica granate} has an important role in folk medicine. \textit{Omegranate} is known as a rich source of pharmacological properties which have been evaluated due to antiparasitic, antibacterial, antifungal, antiproliferative, apoptotic and anti-cancer effects as well as protection against herpes virus, inhibition of LDL oxidation and decrease in atheromatous plaque formation and reduction of systolic blood pressure [18,23,24].

While the vast majority of studies of Microbiology oral relate to various aspects of bacteriology, buccal Protozoology has virtually forgotten, despite the high prevalence of infections in adults where \( T. \) tenax etiologic agent [25].

In this regard there are many publications that reported the complexity of the microbiota residing in dental plaque, not only in quantitative terms but also qualitative including bacteria, fungi, mycoplasma and protoza, among them \( T. \) tenax as part of that system as varied ecological as it is the oral cavity [26,27].

| concentration | 12 hr | 24 hr | 48 hr | 72 hr |
|---------------|-------|-------|-------|-------|
| 12.5          | 98%   | 80%   | 60%   | non motile |
| 25            | 80%   | 70%   | 20%   | non motile |
| 50            | 30%   | 10%   | non motile | non motile |
| 100           | No organism | No organism | No organism | No organism |
| NTC           | 98%   | 85%   | 50%   | non motile |

\( \text{NTC} = \text{Non Treated Culture control} \)

Table 1: Percentage of motility of \textit{T. tenax} after exposure to various concentration of ethanol extract in comparison to normal control.

| concentration | 12 hr | 24 hr | 48 hr | 72 hr |
|---------------|-------|-------|-------|-------|
| 12.5          | 60%   | 50%   | Non motile | No organism |
| 25            | 40%   | 10%   | No organism | No organism |
| 50            | 20%   | No organism | No organism | No organism |
| 100           | No organism | No organism | No organism | No organism |
| NTC           | 100%  | 98%   | 85%   | 50%   |

\( \text{NTC} = \text{Non Treated Culture control} \)

Table 2: Percentage of motility of \textit{T. tenax} after exposure to various concentration of metronidazole in comparison to normal control.
Microscopic observation in the cool of the samples from dental calculus and subgingival plaque by using light microscopy is essential if you want to achieve faster discovery of *T. tenax*, being able to visualize usually with one or two flagella. However, provided the samples should be inoculated in the culture media most appropriate and allow for finding more sure of this species [28,29].

**Conclusion**

The incidence of *T. tenax* was higher in patients in this study suggests that EEPGF might be used as an antiparasitic agent in controlling oral *Trichomonas tenax* infections.

**Acknowledgments**

We thank the Military Academy of Periodontology and Dental Association Clinic staffs and all the participants who shared their time for working on this study.

**References**

1. Wernich DH (1944) Comparative Morphology of the Trichomonad Flagellates of Man. Ann J Trop Med Hyg 54:31: 39-51.
2. el Hayawan IA, Bayoumy MM (1992) The prevalence of Entamoeba gingivalis and *Trichomonas tenax* in periodontal disease. J Egypt Soc Parasitol 22:101-105.
3. Memik F (1968) Trichomonads in pleural effusion. JAMA 204:1145-1146.
4. Sarawaska J, Wojnicz D, Kaczkowski H, Jankowski S (2004) The occurrence of Entamoeba gingivalis and *Trichomonas tenax* in patients with periodontal disease, Immunosuppression and genetic diseases. Adv Clin Exp Med 13:291-297.
5. Vrablic J, Tomnová S, Catár G, Randová L, Suttová S (1991) Morphology and diagnosis of Entamoeba gingivalis and *Trichomonas tenax* in their occurrence in children and adolescent. Bratil Lek Listy 92:241-246.
6. Chiche L, Donali S, Corno G, Benoît S, Granier I, et al. (2005) *Trichomonas tenax* in pulmonary and pleural diseases. Presse Med 34:1371-1372.
7. Mahmoud MS, Rahman GA (2004) Pulmonary trichomoniases improved diagnosis by using polymerase chain reaction targeting *Trichomonas tenax* 18S rRNA gene in sputum specimens. J Egypt Soc Parasitol 34:197-211.
8. Agil F, Khan MS, Owais M, Ahmad I (2005) Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J Basic Microbiol 45:106-114.
9. Parmar HS, Kar A (2008) Medicinal values of fruit peels from *Citrus sinensis*, *Punica granatum* and *Musa paradisiaca* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin, and thyroid hormones. J Med Food 11:376-381.
10. Heber D, Seeram NP, Wyatt H, Henning SM, Zhang Y, et al. (2007) Safety and antioxidant activity of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size. J Agric Food Chem 55:10050-10054.
11. Ricci D, Giamperti L, Bucchini A, Fratemale D (2006) Antioxidant activity of *Punica granatum* fruits. Fitoterapia 77:310-312.
12. Sánchez-Lamar A, Fonseca G, Fuentes JL, Cozzi R, Cundari E, et al. (2007) Assessment of the genotoxic risk of *Punica granatum* (Punicaceae) whole fruit extracts. J Ethnopharmacol 115:416-422.
13. Aviram M, Rosenblat M, Gaitini D, Nitecki S, Hoffman A, et al. (2004) Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. Clin Nutr 23:423-433.
14. Parmar HS, Kar A (2007) Protective role of *Citrus sinensis*, *Musa paradisiaca*, and *Punica granatum* peels against diet-induced atherosclerosis and thyroid dysfunctions in rats. Nutrition Research 27:710-718.
15. Braga LC, Shupp JW, Cummings C, Jett M, Takahashi JA, et al. (2005) Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. J Ethnopharmacol 96:335-339.
16. Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA (2007) Antibacterial activity directed isolation of compounds from *Punica granatum*. J Food Sci 72: M341-M345.

17. Zhang J, Zhan B, Yao X, Gao Y, Shong J (1995) Antiviral activity of tannin from the pericarp of *Punica granatum* L. against genital Herpes virus in vitro. Zhongguo Zhong Yao Za Zhi 20:556-576.
18. Lansky EP, Newman RA (2007) *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 109:177-206.
19. Vasconcelos LC, Sampaio FC, Sampaio MC, Pereira Mdo S, Higino JS, et al. (2006) Minimum inhibitory concentration of adherence of *Punica granatum* Linn (pomegranate) gel against S. mutans, S. mitis and C. albicans. Braz Dent J 17:223-227.
20. Ali NM (2007) In vitro activity of commercially available Egyptian propolis on T. vaginalis. N Egypt J Med 36:7-15.
21. Palmas C, Wakelein D, Gabriele F (1984) Transfer of immunity against Hymenopteran epin nana in mice with lymphoid cells or serum from infected donors. Parasitology 89:287-293.
22. Holez FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, et al. (2002) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 97:1027-1031.
23. Reddy MK, Gupta SK, Jacob MR, Khan SI, Ferreira D (2007) Antioxidant, antimarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. Planta Med 73:461-467.
24. Kim ND, Mehta R, Yu W, Neeman I, Livney T, et al. (2002) Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. Breast Cancer Res Treat 71:203-217.
25. Ferrara A, Conca R, Grassi L, de Carneri I (1986) Possible pathogenic role of *Trichomonas tenax* in chronic periodontitis. Ann Ist Super Sanita 22:253-256.
26. Feki A, Molet B (1990) Importance of *Trichomonas tenax* and *Entamoeba gingivalis* in the human oral cavity. Rev Odontostomatol (Paris) 19:37-45.
27. Mazzilli de Iiia R (1990) Incidence and quantification of mycoplasma in saliva in patients with periodontal disease and subjects with healthy gingiva. Acta Odon tol Venez 28:81-87.
28. Honigberg BM (1978) *Trichomonads* of Importance in human medicine. (2ndedn), Acad Press, New York.
29. Nolte W (1986) Dental Microbiology. (4thedn), Editorial Interamericana, Mexico.