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The Efficacy of Pomegranate (*Punica granatum*) Peel Extract on Experimentally Infected Rats with *Blastocystis* Spp

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Abstract

Here, the study was conducted to evaluate the effect of pomegranate (*Punica granatum*) peel extract on infected rats with *Blastocystis* spp. Anti-protozoan activities were determined by monitoring *Blastocystis* spp. shedding in stools and histopathological changes of intestine of infected rats. Additionally, we evaluated the antioxidant properties of pomegranate peel extract on different groups through measuring the concentration of Malondialdehyde (MDA).

In this work, Punica granatum peel extract-treatment lowered the shedding of cysts very close to nitazoxanide (NTZ) treatment. These data were statistically significant P value ≤ 0.0001. Pomegranate peel extract was found to have the highest anti-lipid peroxidation effect, assessed by measuring MDA level. The inhibitory effect of pomegranate peel extract on lipid peroxidation was significant when compared to NTZ- treated group (P value ≤ 0.0003). As well, histolopathological examination of the intestine showing that Blastocystis spp. were often observed in the infected group without treatment either within the luminal material or at the tip of the epithelium compared to the infected treated groups.

Pomegranate peel extract can be used as alternative therapy for blastocystosis and for developing novel anti Blastocystis drugs. Additionally, these results show clinical evidence that pomegranate peel extract has components act as powerful antioxidants.

Keywords: *Blastocystis* spp; Rats; Pomegranate (*Punica granatum*); MDA

Introduction

Blastocystis spp. is emerging protozoan parasites that inhabit the intestinal tract of humans and many animals with worldwide distribution [1]. Although the pathogenicity of this parasite is controversial [2], antiprotozoan agents are usually administered to the infected individuals [3]. The first choice of chemotherapeutic agent was metronidazole (MTZ) [4]. MTZ was found to be effective against *Blastocystis* spp. in some studies but not in others [5]. Thus, the susceptibility of *Blastocystis* spp. to standard antimicrobials is not clear [3].

Additionally, Rossignol et al. [6] suggested that *Blastocystis* spp. can be treated effectively with nitazoxanide (NTZ), and this therapy was equally effective in both children and adults. Currently antimicrobial resistance among bacteria, viruses, parasites, and other pathological organisms is a serious threat to the infectious disease management worldwide [7]. Considering the side effects of and resistance to many antibacterial drugs, the plant extracts used in traditional medicine are now used as sources for new treatments [8].

The development of drug resistance 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 [9,10]. Plant extracts have shown to be effective against resistant pathogens [11].

Herbal medicine or phytomedicine is now attracting the world's attention as it enhances the health of the body systems without adverse side effects, especially the immune system that protect against pathogens [12].

Punica granatum L, commonly known as pomegranate, is a fruit-bearing deciduous shrub or small tree, native to Asia and belongs to the family Punicaceae [13]. Different parts of the plant such as bark, leaves, immature fruits and fruit rind have medicinal significance [14]. *Punica granatum* is widely employed in various countries as a source of therapeutic agent against a variety of pathogenic microbes [15]. It was utilized as a traditional remedy for thousands of years under the Ayurvedic system of medicine, with extracts from the rind of the fruit and bark of the tree being effective against diarrhea and dysentery [16].

Punica granatum L. is an ancient mystical fruit used in folkloric medicine as a treatment for many diseases such as diarrhea, parasitic worm infections, urinary tract infections, and kidney stones [17,18]. Moreover, many studies indicate that *P. granatum* can slow bacterial growth and inhibit bacterium-induced toxins [19-22]. Furthermore, *P. granatum* peel extract (100 mg/kg) for 10 consecutive days had stimulated immune systems and enhanced cellular immunity in rabbits [23]. Several additional studies have demonstrated the therapeutic effects of *P. granatum* fruit, peel, and juice as powerful antioxidants and anti-inflammatory substances that include polyphenols and tannins [24-31]. *P. granatum* also plays a role in protecting against

cancer diseases [32] and its juice is effective in protecting neuron cells from Alzheimer's disease [33]. The constituents of pomegranate are including high hydrolyzable tannins (punicalins and punicalagins), ellagic acid, a component of ellagitannins, and gallic acid, a component of gallotannins [34]. *P. granatum* has anti-cestodial, anti-nematoidal [35-37], and anti-protozoan activities [8,38,39]. As well, *Punica granatum* peel extract is effective in a murine model of experimental *Cryptosporidium parvum* [40].

Therefore, this study examined the efficacy of aqueous extract of *P. granatum* peel on treatment of *Blastocystis* spp. infection in rats.

Materials and Methods

The study was carried out in the period from January 2016 to May 2016 in the Parasitology Department, Faculty of Medicine, Minia University, Minia, Egypt.

Source of *Blastocystis* isolates

Strains of *Blastocystis* are maintained by repeated subculture with Locke egg (LE) serum medium. The organism could be maintained for more than 3 months [41] in the Parasitology Department, Faculty of Medicine, Minia University Minia, Egypt.

P. granatum materials

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 and stored at 4 °C for the next step [42,43].

P. granatum treatments

Therapeutic doses of *P. granatum* were administered to the animals on day 4 post-inoculation (pi), the day cysts appeared in the feces. *P. granatum* doses of 3 g/kg body weight were prepared fresh (3 g/ml *P. granatum* peel in distilled water).

Animals

Six-week-old male-albino rats weighing 80 gm each were obtained from the experimental house, Faculty of Medicine, Minia University. The animals had free access to standard rodent chow and water. All experimental procedures were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Minia University, Minia, Egypt.

Experimental design

Rats divided into four groups (G1–G4). Each group has six rats: G1: healthy controls, G2: infected/untreated controls G3: infected/ *P. granatum*-treated and G4: infected/ nitazoxanide (NTZ)–treated, from (G2–G4) groups were infected intragastrically with 500 µl LE medium containing 2×10^6 *Blastocystis*. On the fourth day post infection, G3-G4 groups were treated with 500 µl of *P. granatum* doses of 3 g/kg body weight and 500 mg of NTZ [44] respectively administered daily by gastric tubes 1 h before meals for 3 consecutive days [36].

Evaluation of Blastocystis spp. InfeXction in Rats

Detection of cysts shedding in feces

Feces from all rats were examined microscopically at different periods (2,4,6,8, and 10 days) post infection. Quantitative estimation of the infection intensity in the stool samples of *Blastocystis* spp. infected rats was performed according to the method described by [45]. Cysts of *Blastocystis* spp. were counted in at least three fields with estimation of the average number/high power field (HPF) [45].

Histolopathological Examination

On the 10^{th} days post infection, all rats from each group were sacrificed. Tissue samples from walls of small intestine, caecum and colon of scarifying animals were collected then fixed in 10% neutral buffered formalin. The organs were routinely processed and sectioned at 4 to 5 µm thickness. The obtained tissue sections were collected on glass slides, deparafinized and stained with Hematoxylin and Eosin stain. The sections were then examined and observed under light microscope at 10, 40 and 100X magnifications [46,47].

Biochemical Determinations

To assess antioxidant property of *P. granatum* extract, pieces of small intestine were aseptically removed, homogenized, as described by El-Shenawy et al. [48]. Intestinal lipid peroxidation was determined as thiobarbituric acid reacting substance and is expressed as equivalents of malondialdehyde (MDA), using 1,1,3,3-tetramethoxy propane as standard [49].

Statistical Analysis

Data were presented as means ± standard deviation (SD) using Statistical SPSS for Windows, issue 15.8. Statistical significance was determined using t-tests (Man Whitney), Chi-square tests, and one-way analysis of variance. P value less than 0.05 was considered significant.

Results

Shedding of cysts in stool

At day 4 post infection (p.t.) rats in the G2, G3 and G4 shed variable cysts in feces. The shedding of cysts varied in the range between 10-11/HPF (Table 1). On the 6th day p.t., the cyst shedding output differed between pomegranate peel and NTZ-treated groups and non-treated group (Table 1). Pomegranate peel and NTZ-treated groups significantly lowered the shedding of cysts. These data were statistically significant (Table 1).

Biochemical determinations

Rats infected with Blastocystis exhibited increased levels of MDA production (118.85 ± 0.9 *vs.* 50.93 ± 0.54, $P \leq 0.000005$) compared to that of uninfected controls. Treatment of infected rats with Punica granatum, and NTZ reduced MDA levels 30.07 ± 5.9 and 61.01 ± 1.1, respectively, as shown in (Table 2).

Rat group/parasite load Days (post infection)	

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Parasite load in the stool of rats of different studied groups (Mean ± SD)						
Healthy group	0	0	0	0	0	0
Infected untreated	0	0	11.4 ± 0.9	**15.25 ± 0.9	***18.25±0.9	***23.6±1.4
Infected (+ *P. granatum)*	0	0	11.6±0.5	7.2±0.8	3.4±0.5	1.4±0.5
Infected (+ NTZ)	0	0	10.5±0.5	6.2±0.8	2.4±0.5	0.9±0.4
*: P value: ≤ 0.05, **: P value: ≤ 0.001; ***: P value: ≤ 0.0001.						

Table 1: Effect of *P. granatum* peel on the cyst's shedding intensity in the stool of rats of the various studied groups. **: P value between infected untreated rats and infected (+*P. granatum*) and /or infected (+ NTZ) rats.

MDA (NM/g)	**Non-infected**	**Infected**	**Infected (+(*P. granatum*)**	**Infected (+ NTZ)**
	50.93±0. 54	118.85±0.9	30.07±.5.9	61.01±1.1
p. value			0.0003**	0.03**
**: P value between infected untreated rats and infected (+*P. granatum*)and /or infected (+ NTZ) rats; *: P value between non- infected and infected rats.				

Table 2: Effect of *P. granatum* peel on the level of malondialdehyde, in the intestine of rats infected with *Blastocystis* spp.

Histopathology

In intestinal H&E histological sections, *Blastocystis* was frequently observed within the luminal material (Figures 1A and 2A) in the infected untreated group. On the other hand, very few organisms were observed within the luminal material of the infected treated groups (Figures 1B and 2B). Associated pathology was in the form of some epithelial damage and mucosal sloughing in the infected untreated group (Figure 1A) compared to the infected treated groups (Figure 1B). Furthermore, the infected untreated group showed also exhibited much goblet cell hyperplasia (Figure 3A) compared to that of the infected treated ones (Figure 3B).

Discussion

In the current study a rat model of *Blastocystosis* was used to determine the efficacy of aqueous *P. granatum* peel extract as a treatment for *Blastocystis* spp infections. Cyst shedding, and histological changes are useful for determining the pathology of *Blastocystis*spp. infections [50]. Therefore, we examined these parameters during the course of *Blastocystis*spp infections with and without *P. granatum* treatment.

In the recent years, the use of plants with preventive and therapeutic effects contributes to health care needs [51]. There are three main reasons for interest in the treating and healing power of plant extracts. 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, *P. granatum* has an important role in folk medicine. Pomegranate 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 plaque formation and reduction of systolic blood pressure [27,34,43].

Blastocystis-infected rats that were treated with *P. granatum* peel in this study showed a complete elimination in fecal cyst shedding by day 10 pi. The reduction and elimination of fecal cyst shedding in response to *P. granatum* treatment seen here may be attributable to the presence of metabolic toxins which have a direct effect on parasite growth in the intestines, the production of the sexual phases, and/or the formation of cysts.

We previously mention that the constituents of pomegranate are including tannins (punicalins and punicalagins), ellagic acid, and gallic acid [34]. It was reported that ellagic acid has anti-microbial activity and punicalagin has anti-food-borne pathogens [52,53].

Furthermore, *P. granatum* peel contains major phenolic compounds, such as organic acids [22,31],that can directly inhibit*Blastocystis*infections. Organic acids have an attenuating effect on the growth of enteropathogenic microbes [54,55]. Specifically, organic acids have inhibitive effects on *Blastocystis* infections [56] and can reduce parasite vitality [57]. Additionally, the hydroxyl group of the phenolic compounds in P. granatum can increase toxicity against all organisms [22,58].

Moreover, *P. granatum* decreased MDA production significantly as Sing et al. [59] suggested that Pomegranate ability to suppress hydroxyl radicals is directly related to the prevention of propagation lipid peroxidation thus reducing the rate of chain reaction and the peel extract has the higher antioxidant activity than seeds extract. In addition, it has been reported that, several *P. granatum* metabolites includes polyphenols and tannins which acts as an antioxidant by scavenging reactive oxygen species (ROS), preventing lipid oxidation and production of pro-inflammatory messengers. [24-31]. Also, Sing et al. [59] reported that the Pomegranate ability to suppress hydroxyl radicals seems to be directly related to the prevention of propagation lipid peroxidation thus reducing the rate of chain reaction.

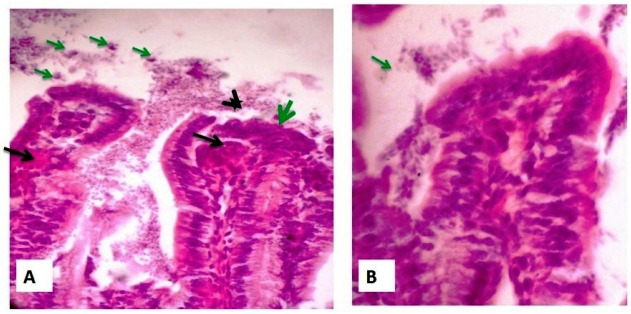

Figure 1 :(A) Photomicrograph of distal ileal section of infected untreated rats shows some preserved villous pattern. The core is infiltrated with few chronic inflammatory cells. The surface is partially ulcerated (40X). (black arrow head). There are areas of edema and congestion of blood vessels (black arrow). The lumen is filled with necrotic debris admixed with mucin and several forms of *Blastocystis*, vacuolar and granular (green arrows) (40X). **(B)** Photomicrograph of distal ileal section of infected treated rats either with Punica granatum, and/or NTZ show intact mucosa of ileocecal area (40X). Very few chronic inflammatory cells are presented in the core. There are very few forms of *Blastocystis* (green arrows) in the lumen.

Based on the observations in the study, it is possible that *P. granatum* is able to activate or desuppress the anti-parasite associated gene(s) in mammals. At the molecular level, *P. granatum* may reduce the level of DNA methylation at promoters of anti-parasite gene(s) and facilitate their expression. As DNA methyltransferases (DNMTs) and some histone methyltransferase (HMTs) have been shown to play a role in the maintenance of DNA methylation globally and loci specifically [60-62] *P. granatum* may cause the promoter hypomethylation via affect the function of DNMTs or HMTs. Therefore, to fully understand how does *P. granatum* eliminate *Blastocystis* spp, it is interesting to investigate whether *P. granatum* plays a role in the regulation of epigenetic modifiers in mammals.

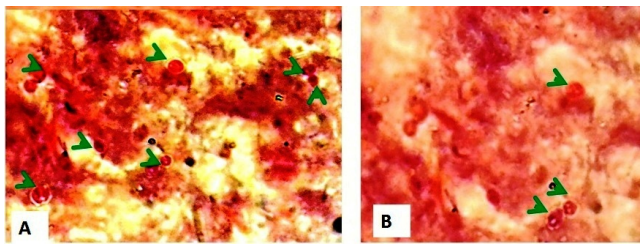

Figure 2: (A) Photomicrograph demonstrates *Blastocystis* in intestinal lumen of infected untreated rats shows several forms of *Blastocystis*, vacuolar and granular (100X) (green arrows head). **(B)** Photomicrograph of intestinal lumen of infected treated rats either with *Punica granatum*, and/ or NTZ shows very few parasites (100X) (green arrows head).

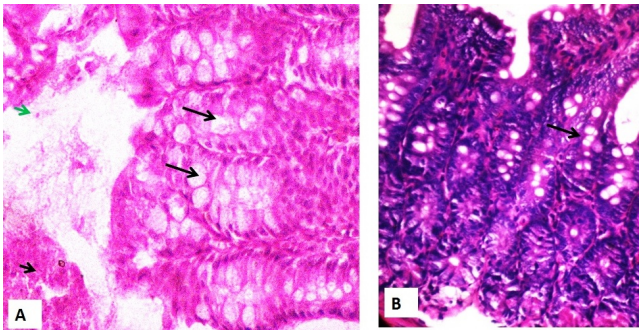

Figure 3: (A) Photomicrograph of cecal section of infected untreated rats shows severe infiltration of the mucosa with chronic inflammatory cells. The mucosal glands are partially destroyed and shows goblet cell hyperplasia (40X) (black arrow). The surface is partially ulcerated. The lumen is filled with necrotic debris mixed with mucin and the organism (green arrows). (B) Photomicrograph of cecal section of infected treated rats shows complete regeneration of the mucosal glands with massive reduction of the inflammatory cell infiltrate. Moderate amount of goblet cells are detected (black arrow). The core is infiltrated with few chronic inflammatory cells. The surface shows no ulceration. The lumen is clear (40X).

References

1. Stensvold CR, Lewis HC, Hammerum AM, Porsbo LJ, Nielsen SS, et al. (2009) Blastocystis: unraveling potential risk factors and clinical significance of a common but neglected parasite. Epidemiol Infect 137: 1655–1663.
2. Yakoob J, Abbas Z, Beg MA, Naz S, Awan S, et al. (2011) In vitro sensitivity of Blastocystis hominis to garlic, ginger, white cumin, and black pepper used in diet. Parasitol Res 9: 379–385.
3. Moghaddam D, Azami M, Ghadirian E (2005) Blastocystis hominis and the evaluation of efficacy of metronidazole and trimethoprim/ sulfamethoxazole. Parasitol Res 96: 273–275.
4. Coyle CM, Varughese J, Weiss LM, Tanowitz HB (2012) Blastocystis treat or not to treat. Clin Infect Dis 54: 105–110.
5. Nigro L, Larocca L, Massarelli L, Patamia I, Minniti S, et al. (2003) A placebo-controlled treatment trial of Blastocystis hominis infection with metronidazole. J Travel Med 10: 128–130.
6. Rossignol JF, Kabil SM, Said M, Samir H, Younis AM (2005) Effect of nitazoxanide in persistent diarrhea and enteritis associated with Blastocystis hominis. Clin Gastroenterol Hepatol 3: 987–991.
7. Sosa AJ, Byarugada DK, Amábile-Cuevas CF, Hsueh PR, Kariuki S, et al. (2010) Antimicrobial resistance in developing countries. Springer New York 554.
8. Calzada F, Yepez-Mulia L, Aguilar A (2006) In vitro susceptibility of Entamoeba histolytica and Giardia lamblia to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 108: 367–370.
9. Aqil F, Khan MS, Owais M, Ahmad I (2005a) Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant Staphylococcus aureus. J Basic Microb 45: 106-114.
10. 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.
11. Aqil F, Khan MS, Owais M, Ahmad I (2005b) Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant Staphylococcus aureus. J Basic Microb 45: 106–11.

12. Pandy M, Debnath M, Gupta S, Chikara SK (2011) Phytomedicine: An ancient approach turning into future potential source of therapeutics. J Pharmacognosy and Phytotherapy 3: 27–37.
13. Qnais EY, Elok AS, Abu Ghalyun YY, Abdulla FA (2007) Antidiarrheal Activity of the Aqueous Extract of Punica granatum (Pomegranate) Peels. Pharmaceut Bio 45:715–720.
14. Arun N, Singh DP (2012) Punica granatum: a review on pharmacological and therapeutic properties. IJPSR 3: 1240–1245.
15. Vijayanand S, Hemapriya J (2011) In vitro Antibacterial Efficacy of Peel and Seed Extracts of Punica granatum against Selected Bacterial Strains. Int J Med Res 1: 231–234.
16. Jayaprakasha GK, Negi PS, Jena BS (2006) Antimicrobial activities of pomegranate. In: Seeram. Pomegranates: Ancient Roots to Modern Medicine. New York, NY, USA: CRC Press. 168.
17. Navarro V, Villarreal LMA, Rojas G, Lozoya X (1996) Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J Ethnopharmacol 53: 143–147.
18. Sudheesh S, Vijayalakshmi NR (2005) Flavonoides from Punica granatum–potential antiperoxidative agents. Fitoterapia 76:181–186.
19. Braga LC, Shupp JW, Cumings C, Jett M, Takahashi JA, et al. (2005) Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. J Ethnopharmacol 96: 335–339.
20. Ghosh A, Das B, Roy A, Mandal B, Chandra G (2008) Antibacterial activity of some medicinal plant extracts. J Nat Med 62: 259–262.
21. Bialonska D, Kasimsetty S, Schrader K, Ferreira D (2009) The effect of pomegranate (Punica granatum L.) by products and ellagitannins on the growth of human gut bacteria. J Agric Food Chem 57: 8344–8349.
22. Choi J, Kang O, Lee Y, Che H, Oh C, et al. (2011) In Vitro and in vivo antibacterial activity of Punica granatum peel ethanol extract against Salmonella. Evid Based Complement Alternat Med 1– 8.
23. Gracious RR, Selvasubramanian S, Jayasundar S (2001) Immunomodulatory activity of Punica granatum in rabbits-a preliminary study. J Ethnopharmacol 78: 85–87.
24. Aviram M, Dornfeld L, Roseblat M, Volkova N, Kaplan M, et al. (2000) Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr 71: 1062–1076.
25. Aviram M, Dornfeld L (2001) Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. Atherosclerosis. 158: 195–198.
26. Aviram M, Dornfeld L, Kaplan M, Coleman R, Gaitini D, et al. (2002) Pomegrante juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drug Exp. Clin Res 28: 49–62.
27. Kim ND, Mehta R, Yu W, Neeman I, Livney T, et al. (2002) Chemo preventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. Breast Cancer Res 71: 203–217.
28. Cerda B, Llorach R, Ceron J, Espin JC, Tomas-Barberan FA (2003) Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. Eur J Nutr 42: 18–28.
29. Suzuki T, Hashimoto T, Yabu Y, Kido Y, Kimitoshi K, et al. (2004) Direct evidence for cyanide-insensitive quinol oxidase (alternative oxidase) in apicomplexan parasite Cryptosporidium parvum: phylogenetic an therapeutic implications. Biochem Biophys Res Commn 313:1044–1052.
30. Afaq F, Saleem M, Krueger CG, Reed JD, Mukhtar H (2005) Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF–kappa B pathways and inhibits skin tumoriogenesis in CD-1 mice. Int J Cancer 113: 423–433.
31. Gasemian A, Mehrabian S, Majd A (2006) Peel extracts of two Iranian cultivars of pomegranate (Punica granatum) have antioxidant and antimutagenic activities. Pak J Biol Sci 9: 1402–1405.
32. Syed DN, Afaq F, Mukhtar H (2007) Pomegranate derived products for cancer chemoprevention. Semin Cancer Boil 17: 377–385.
33. Wang C, WuY, Qin J, Sun H, He H (2009) Induced susceptibility of host is associated with an impaired antioxidant system following infection with Cryptosporidium parvum in Se-deficient mice. PLoS One 4:e4628.
34. Reddy MK, Gupta SK, Jacob MR, Khan SI, Ferreira D (2007) Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from P. granatum L. Planta Med 73: 461–467.
35. Prakash V, Singhal KC, Gupta RR (1980) Anthelmintic activity of Punica granatum and Artemisia siversiana. Indian J Pharmacol 12: 61A–80A.
36. Akhtar MS, Riffat S (1985) Efficacy of Punica granatum, Linn. (ANAR) fruit (Rinds) against naturally acquired nematodal and cestodal infections in sheep. J Pharm Pb Univ Lhr 6: 17–24.
37. Korayem AM, Hasabo SA, Ameen HH (1993) Effects and mode of action of some plant extracts on certain plant parasitic nematodes. Anz Schadl 66: 32–36.
38. Dell'Agli M, Galli G, Corbett Y, Taramelli D, Lucantoni L, et al. (2009) Antiplasmodial activity of Punica granatum L. fruit rind. J Ethnopharmacol 125: 279–285.
39. El-Sherbini GT, El Gozamy BR, Abdel-Hady NM, Morsy TA (2009) Efficacy of two plant extracts against vaginal trichomoniasis. J Egypt Soc Parasitol 39: 47– 58.
40. Al-Mathal EM, Alsalem AM (2012) Pomegranate (Punica granatum) peel is effective in a murine model of experimental Cryptosporidium parvum. Exp Parasitol 131: 350–357.
41. Saksirisampant W, Nuchprayoon S, Pradniwat P, Lamchuan D (2010) Boeck and Drbohlav Locke egg serum medium for detection of Blastocystis hominis. Chula Med J 54:527–536.
42. 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.
43. Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA (2007) Antibacterial activity directed isolation of compounds from Punica granatum. J Food Science 72: 341–345.
44. White CA (2004) Nitazoxanide: a new broad spectrum antiparasitic agent. Expert Rev Anti Infect Ther 2:43–49.
45. Shlim DR, Hoge CW, Rajah R, Rabold JG, Echeverria P (1995) Is Blastocystis hominis a cause of diarrhea in travelers? A prospective controlled study in Nepal. Clin Infect Dis 21: 97–101.
46. Moe KT, Singh M, Howe J, Ho LC, Tan SW, et al. (1997) Experimental Blastocystis hominis infection in laboratory mice. Parasitol Res 83: 319–325.
47. Bancroft JD, Gamble A (2008) Theory and practice of histological techniques. Churchill- Livingstone, Edinburgh, London, Melbourne, New York.
48. El-Shenawy NS, Soliman MF, Reyad SI (2008) The effect of antioxidant properties of aquatic garlic extract and Nigella sativa as anti-schistosomiasis agent in mice. Rev Inst Med trop Sao Paulo 50: 29– 36.
49. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52: 302– 31.
50. Guitard J, Menotti J, Desveaux A, Alimardani P, Porcher R, et al. (2006) Experimental study of the effects of probiotics on Cryptosporidium parvum infection in neonatal rats. Parasitol Res 99: 522–527.
51. Holetz 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: 1027–31.
52. Taguri T, Tanaka T, Kouno I (2004) Antimicrobial activity of 10 different plant polyphenols against bacteria causing food- borne disease. Biol Pharm Bull 27: 1965–1969.
53. Thiem B, Goślińska O (2004) Antimicrobial activity of Rubus chamaemorus leaves. Fitoterapia 75: 93–95.
54. Anderson ME (1992) Efficacies of acetic, lactic and two mixed acids in reducing numbers of bacteria on surfaces of lean meat. J Food Safety 12: 139–147.

55. Hsiao CP, Siebert KJ (1999) Modeling the inhibitory effects of organic acids on bacteria. Int J Food Microbiol 47: 189–201.
56. Watarai S, Tana T, Koiwa M (2008) Feeding activated charcoal from bark containing wood vinegar liquid (nekka-rich) is effective as treatment for cryptosporidiosis in calves. J Dairy Sci 91: 1458–1463.
57. Kniel KE, Sumner SS, Lindsay DS, Hackney CR, Pierson MD, et al. (2003) Effect of organic acids and hydrogen peroxide on Cryptosporidium parvum viability in fruit juices. J Food Protect 66: 1650–1657.
58. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564–565.
59. Sing RP, Chidambara Murthy KN, Jayaprakasha GK (2002) Studies on the Antioxidant Activity of Pomegranate (Punica granatum) Peel and Seed Extracts Using in Vitro Models. J Agric Food Chem 50 : 81–86.
60. Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 12:69:915-26.
61. Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 29:99:247-57.
62. Zhang T, Termanis A, Özkan B, Bao XX, Culley J, et al. (2016) G9a/GLP Complex Maintains Imprinted DNA Methylation in Embryonic Stem Cells. Cell Rep 5:15:77-85.