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RESEARCH ARTICLE

Evaluation of the Effectiveness of Olive (*Olea europaea*) Leaf and Pomegranate (*Punica granatum*) Flower Extracts in the Viability of the Protoscolices of *Echinococcus granulosus In Vitro*

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Abstract

The present study was conducted for the period from 1/10/2017 up to 1/3/2018 at the Department of Biology - College of Education for Girls-Kufa University. It aims to assess the effectiveness of ethanolic extracts for olive (*Olea europaea*) leaf and pomegranate (*Punica granatum*) flower in the viability of protoscolices of *Echinococcus granulosus* parasite *in vitro*. The results demonstrated that the ethanolic extracts for olive leaf and pomegranate flower are very effective in the devastation of the protoscolices, and that the mortality rate increases with concentration and duration of exposure to both extracts .The results showed that the ethanolic extracts for olive leaf was more powerful than the ethanolic extracts for pomegranate flower in the mortality of protoscolices, where the LC50 for the mortality of the protoscolices was (9.7) mg/ml and (11.1) mg/ml, respectively, following 15 minutes of exposure to the extracts, while the LC50 for the mortality of the protoscolices (5.9) mg/ml and (6.6) mg/ml respectively, after 60 minutes of exposure to the extracts. The results also showed that the ethanolic extract for olive leaf led to the decimation of the protoscolices in less time than the ethanolic extract for pomegranate flower when exposed to the concentration of (4) mg / ml, where the LT50 of the protoscolices of (44.6) minutes and (79.4) minutes, respectively, while exposed to the concentration of (16) mg/ml, where the LT50 of the protoscolices of (15.7) minutes and (18.3) minutes, respectively. The conclusion from this study that leaves of olive plant and pomegranate flowers as natural materials can be used to kill the protoscolices of the *E. granulosus* parasite before a surgical procedure to remove the hydatidcysts .

Keywords: *Olive leaf, Pomegranate flower, Ethanolic extracts, Protoscolices, Echinococcus granulosus, In vitro.*

Introduction

Hydatidosis is AA chronic disease of medicinal and veterinary significance that causes the worldwide *Echinococcus granulosus* to spread in numerous parts of the world, particularly in areas where livestock are raised.The infection is endemic in Asia, North Africa, South, Central and North America, Canada and the Mediterranean regions, and diseaseis common in ruralregions where there is close contact between individuals [1].

Mutts are the last has, since the grown-up tapeworm connects to the digestive organs of the puppy's digestive organs and multiplies, prompting egg arrangement. The middle of the road has, for example, Human, and in addition household steers, including sheep, cows, camels and pigs, end up tainted after ingestion of eggs through direct contact with puppies or in a roundabout way through sustenance, water or soil defiled with worm eggs. The freed developing lives infiltrate the intestinal divider and through the framework my gut essentially enters the liver by 50-70% and the lungs by 20-30% or whatever other organs, where the hydatid sores develops and amplify [2]. Obstructive jaundice, stomach torment, burst of the bile, and jaundice result from weight caused by the sore on the liver [3].

Optional diseases happen through the development of protoscolices in the peritoneum, pleura, bronchial tubes and bile pipes after the burst of the hydatid growths [4].Hydatid pimple ailment has general wellbeing significance in endemic zones, as well as in nations where malady isn't endemic because of transitory movement and trade of animals that make new zones of settlement [5, 6].

Surgical expulsion of hydatid growths is as yet favored in numerous parts of the world, likewise, chemotherapy with benzimidazole subordinates, Albendazole and Mebendazole, and in addition PAIR, which implies Puncture, Aspiration, Injection, and Re-goal, is prescribed as elective surgeries, particularly for patients who don't have intricacies [7].

So as to decrease the danger of spillage of pimple substance (protoscolices) amid surgery and the repeat of the development of the blisters and optional diseases saw in around 10% of the surgical strategy, so the utilization of deadly substances for the protoscolices is vital [1]. The lthal substances of the protoscolices which at present exists Include: Hydrogen peroxide [8], Chlorhexidine [9], Formalin[10], Absolute ethanol [11] and Hypertonic saline [12], that are related with negative impacts, for example, sclerosing cholangititis and liver necrosis [13,14].

And in addition the rise of protection from different medications by pathogenic microorganisms to people because of the aimless utilization of antimicrobial business drugs utilized as a part of the treatment of irresistible ailments and consequently requires the scan for new mixes of normal items and improvement as hostile to pathogens, including the protoscolices of E. granulosus parasite for having a couple of symptoms and ease and exceedingly effective [15, 16]. Characteristic items have opened the best approach to new treatments and to understanding a considerable lot of the biochemical and atomic pathways and cell science [17].

The olive tree (Olea europaea L.) is a hallowed plant that God swore in the Holy Quran [18]. The olive tree is developed in numerous parts of the world, yet the Mediterranean it is the primary place to develop, which speaks to almost 98% of horticulture everywhere throughout the world. Aside from the Mediterranean, the olive plant generally developed in the Arabian Peninsula, India and Asia [19]. Olive leaves contain phenolic mixes and the most rich mixes officially recognized in olive leaf removes are. Oleuropein, Hydroxytyrosol, Verbascoside Apigenin-7- glucoside and Luteolin-7- glucoside. Olive leaf extricate has been broadly utilized as a part of society drug to battle fever and different illnesses, for example, Malaria [20]. Numerous specialists have recorded that olive leaf separate has antimicrobial adequacy because of its high substance of phenolic mixes [21-25]. The pomegranate plant is one of the most seasoned known organic product plants said in the Holy Quran, Bible, Torah and the Babylonian Talmud. Its development spread to Persia and from that point spread to North Africa and the Mediterranean areas [26].

Pomegranate bark and roots were utilized to treat parasitic contaminations and dry blooms were utilized to treat bronchitis and wicked loose bowels. Pomegranate was additionally utilized as a part of the treatment of diabetes in antiquated Greek prescription [27-29]. Antibacterial impacts of watery, ethanolic and acetonic concentrates of pomegranate were seen by various scientists [30, 31].

Because of the therapeutic significance of olive leaves and pomegranate blossoms and their antimicrobial properties, along these lines, this examination, which means to assess the adequacy of ethanolic separates for olive (Olea europaea) leaf and pomegranate (Punica granatum) bloom in the suitability of protoscolices of Echinococcus granulosus parasite In vitro.

Materials & Methods

The flow consider was led for the period from 1/10/2017 until 1/3/2018 in the Department of Biology - Faculty of Education for Girls - University of Kufa, where the leaves of the olive plant were gathered from the olive trees situated in the Faculty of Education for young ladies and washed the leaves with clean water and left in the research facility to dry after cut into little pieces and worked by electric processor to obtain leaves powder. Blend (10) g of powder with 200 ml of ethyl liquor (70% volume).

The blend was mixed by thermo-shaker for two hours at a temperature of 38 ° C. The examples were separated by centrifugation at 5000 cycles for every moment, after which the concentrate was dried by rotational evaporator at a temperature of 38 ° C. The dry concentrates were then put away in dim compartments at a temperature of 4 ° C until utilize [25]. The pomegranate blossoms were gotten from the nearby markets of Najaf area, where the blooms were cut into little pieces and pulverized by the electric processor to obtain the flower powder.

Consolidate 10 g of botanical powder with 50 ml of ethyl liquor 70% (volume/volume) in a 100 ml glass flagon. Cover and leave the flagons at lab temperature and in a dull place for 24 hours with blending utilizing Shaker to guarantee that the substance is presented to liquor. The examples were centrifuged at 5000 cycles for each moment, after which the concentrate was dried by a rotating evaporator at a temperature of 38 ° C. The dry concentrates were then put away in dull holders and at a temperature of 4 ° C until utilize [32].

Tests of sheep's livers, which are normally tainted with hydatidosis, were gathered from the Najaf territory slaughter and were set in clean holders. They were exchanged to the postgraduate research center in the Department of Biology - Faculty of Education for Girls - University of Kufa. The planning and forgetting about methods were conveyed in a period not surpassing two hours, where the external surface of the blister was cleaned by 70% ethyl liquor and 75% of the hydatid liquid was pulled and put in a sterile glass holder and after that evacuated created layer and set in a glass dish and washed with clean saline arrangement 0.9% for three times.

Utilize the axis at 3000 rpm for three minutes to accelerate the protoscolices in the hydatid liquid and the wash arrangement of the producing layer, where the leachate was disregarded and suspended the precipitation containing the primates by 0.9% saline arrangement. The aggregate number of protoscolices was figured utilizing a settled size exchange technique utilizing a miniaturized scale pipette. The aggregate number of protoscolices was estimated in 10 µl of the suspension utilizing the compound magnifying instrument under 20X and the quantity of three duplicates was ascertained to figure the aggregate number of protoscolices.

Analyze the feasibility of the protoscolices utilizing 0.01% eosin color, where break even with measures of the suspension of protoscolices were blended with the eosin color, inspected under a 20X magnifying instrument and utilized a manual meter. A sum of 200 protoscolices of living and dead protoscolices are checked, where the living protoscolices show up in a greenish shading, while the dead protoscolices seem red [33].

Set up a stock answer for each concentrate by dissolving 2 g of dry concentrate in 100 ml of 0.9% saline arrangement and after that the centralizations of 4, 8 and 16 mg/ml for each concentrate were arranged and after that kept at a temperature of 4 ° C until the point when they were utilized as a part of protoscolices reasonability explore tests in vitro. The suspension is all around shaked keeping in mind the end goal to regularize the dispersion of protoscolices in suspension. The aggregate number of protoscolices was figured in 1 milliliters of suspension utilizing the settled size exchange technique.

Twelve test tubes with a tight top were utilized, with three tubes for every fixation and control for each concentrate, and afterward exchange (1) milliliters of protoscolices suspension containing around 2000±20 protoscolex. The tubes containing the protoscolices were treated with centralizations of 4, 8, and 16 mg/ml for each concentrate. The control tubes were treated with 0.9% saline arrangement. The practicality of protoscolices were figured utilizing 0.01% eosin color at interims of 15, 30 and a hour after treatment. (200) of living and dead protoscolices were tallied, where the living protoscolices show up in the a greenish shading, while the dead protoscolices seem red [33].

Statistical Analysis

The outcomes were measurably dissected utilizing the ANOVA table and the minimum critical contrast (L.S.D) was utilized as a part of the finding of factual contrasts between medications [34]. The technique for minimum squares for esteem deviation was connected to figure the deadly fixation for 50 (LC50) and deadly time for 50 (LT50) of the protoscolices and the relapse coefficient (r) was computed [35].

Results & Discussion

The consequences of the present investigation appeared in Table (1) and (2), demonstrated that the ethanolic concentrates of the olive leaves and pomegranate blooms were extremely powerful in the obliteration of the protoscolices, and that the mortality rate increments consistently with expanding the fixation and term of introduction to the two concentrates. There was a noteworthy relationship coefficient at 0.05 between the focus and the mortality and between the time

and the mortality of the protoscolices as in the Table (3) and (4). The mortality rate, when the treatment with 4 mg/ml of the ethanolic concentrates of olive leaves and pomegranate blooms, was 34.2 % and 26.6 % , individually, following 15 minutes of presentation to separates, while the focus 16 mg/ml giving mortality rate 97.6 % and 91.7 % individually following a hour of introduction to removes. Measurable investigation demonstrated that there are noteworthy contrasts between the mortality rate for the demolition of protoscolices in medicines and control at the level of likelihood of 0.05.

Table 1: The impact of ethanolic concentrate of olive leaves in the protoscolices of Echinococcus granulosus parasite

Concentration (mg / ml)	Mortality percentage of the protoscolices in the time periods (minute)		
	15	30	60
Control	0a	0a	0a
4	34.2b	46.7b	52.8b
8	45.7c	57.2c	77.9c
16	53.3d	72.4d	97.6d
L.S.D at the level 0.05	6.4	8.1	9.1

There were statistically significant differences at the level of significance of 0.05 between the rates that bear different letters of vertical comparisons, while there were no statistically significant differences between the rates bearing similar letters.

Table 2: The impact of ethanolic concentrate of pomegranate blooms in the protoscolices of Echinococcus granulosus parasite

Concentration (mg / ml)	Mortality percentage of the protoscolices in the time periods (minute)		
	15	30	60
Control	0a	0a	0a
4	26.6b	37.2b	45.4b
8	35.8c	46.9c	70.5c
16	43.7d	66.8d	91.7d
L.S.D at the level 0.05	5.1	7.1	10.2

There were statistically significant differences at the level of significance of 0.05 between the rates that bear different letters of vertical comparisons, while there were no statistically significant differences between the rates bearing similar letters

Additionally the outcomes appeared, the ethanolic concentrate of olive leaves was more powerful than the ethanolic concentrate of pomegranate blossoms in the devastation of the protoscolices, where the LC50 of the protoscolices was 9.7mg/ml and 11.1mg/ml, separately, following 15 minutes of presentation to the concentrates, while the LC50 of the protoscolices was 5.9 mg/ml and 6.6 mg/ml, individually, following a hour of introduction to the concentrates (Table 3). The outcomes likewise demonstrated that the ethanolic concentrate of the olive leaves brought about the annihilation of the protoscolices in less time than the ethanolic concentrate of the pomegranate blooms. The LT50 of the protoscolices was 44.6 and 79.4 minutes, individually, at a convergence of 4 mg/ml, while at the focus 16 mg/ml, the LT50 of the protoscolices was 15.7 minutes and 18.3 minutes separately (Table 4).

Table 3: LC50 of the protoscolices for Echinococcus granulosus parasite

Time (minute)	LC50 (mg / ml) of the protoscolices when exposed to ethanolic extracts			
	Olive leaves	Correlation coefficient (r *)	Pomegranate flowers	Correlation coefficient (r *)
15	9.7	0.915	11.1	0.918
30	7.9	0.924	8.8	0.930
60	5.9	0.969	6.6	0.960

*Significant levels at 0.05

Table 4: LT50 of the protoscolices for *Echinococcus granulosus* parasite

Concentration (mg/ml)	LT50 (minutes) of the protoscolices when exposed to ethanolicextracts			
	Olive leaves	Correlation coefficient (r *)	Pomegranate flowers	Correlation coefficient (r *)
4	44.6	0.977	79.4	0.991
8	19.2	0.949	28.6	0.977
16	15.7	0.963	18.3	0.978

*Significant levels at 0.05

The larger part of concentrates on the adequacy of ethanolic concentrate of olive leaves against microbial and parasitic microorganisms. [36] examined the impact of CH3)2CO, ethyl liquor and ethyl acetic acid derivation concentrates of the olive leaves in the development of various parasites, and found that the CH3)2CO removes are the

best in repressing the development of organisms Saccharomyces uvarum and Candida oleophila [23].Recorded that the olive leaf separates have hostile to microbial movement in vitro. Bacillus Cereus and Candida albicans were the most delicate to the concentrates and the minimum touchy were the Pseudomonas aeruginosa microscopic organisms.

The viability of olive concentrates was because of the nearness of the viable phenolic mixes of Oleuropein and antimicrobial instrument is contort of proteins and influence the penetrability of the cell film, and [37] demonstrated that the blend of phenol mixes arranged from olive leaf remove indicated inhibitory impacts against Bacillus cereus and Salmonella enteritidis [38].

Examined the impact of fluid and ethanolic concentrates of O. europaea and Satureja khuzestanica leaves against protoscolices of E. granulosus in vitro. The concentrates of leaves of the olive plant with groupings of 0.1% and 0.01% were observed to be exceptionally powerful in killing the protoscolices following 120 minutes of presentation, while S. khuzestanica separates with 0.1% focus had a noteworthy impact in killing the protoscolices in 30, 60 and 120 minutes of presentation.

As indicated by the examination by [39], the concentrate of olive leaves was higher powerful against Bacillus cereus, while the most reduced hostile to bacterial impact against Salmonella typhimurium, and [40] removed the phenolic mixes from olive leaves by 80% ethyl liquor. The entire hindrance of foodborne pathogens was discovered when utilizing the focus 62.5 mg/ml. Because of the expanding improvement of medication protection and the undesirable impacts of existing antimicrobials bringing about the scan for new antimicrobial mixes was the focal point of various examinations [41]. Enthusiasm for characteristic items has expanded as of late. Restorative plants have been recognized as wellsprings of organically dynamic mixes, were secluded and broke down to decide instruments and target areas [42, 30].

Researched the antimicrobial viability of six assortments of pomegranate, and called attention to that the adequacy of pomegranate, because of containing successful mixes, for example, add up to phenolic mixes and anthocyanin exacerbates that have an inhibitory impact of gram positive and negative microorganisms and Candida albicans [43].

Examined the impact of alcoholic concentrates of pomegranate blooms on the bacterial pathogens of Streptococcus sanguinis, Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus, and Enterococcus faecalis. These concentrates were found to significantly affect all bacterial oral pathogens, Streptococcus mutans are the touchiest to extricate. The conclusion from this examination that leaves of olive plant and pomegranate blossoms as regular materials can be utilized to murder the protoscolices of the E. granulosus parasite before surgery to expel the water packs.

References

1. McManus DP, Zhang W, Li J, Bartley PB (2003) Echinococcosis. Lancet, 362:1295-1304.
2. Eckert J, Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev., 17:107-135.
3. Marti BL, Menor SF (1990) Complications of hepatic hydatid cysts: ultrasound, computed tomography, and magnetic resonance diagnosis. Gastrointest. Radiol., 15: 119-125.
4. Ersan O, Yusuf B (2002) IntrabilIary obstruction due to ruptured hepatic hydatid cyst: evaluation with computed tomography and magnetic resonance imaging. J. Clin. Gastroenterol., 35:2-16.
5. Mamuti W, Yamasaki H, Sako Y, Nakaya K, Nakao M, Lightowlers MW, Ito A (2002) Usefulness of hydatid cyst fluid of Echinococcus granulosus developed in mice with secondary infection for serodiagnosis of cystic echinococcosis in humans. Clin. Diagn. Lab. Immunol., 9:573-576.
6. Frider B, Larrieu E (2010) Treatment of liver hydatidosis: How to treat an asymptomatic carrier? World J. Gastroenterol., 16:4123-4129.
7. Junghanss T, da Silva, AM Horton, J Chiodini, PL Brunetti E (2008) Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. Am. J. Trop. Med. Hyg., 79:301-311.
8. Ali BH, Blunden G (2003) Pharmacological and toxicological properties of Nigella sativa. Phytother. Res., 17:299-305.

9. Randhawa MA, Al-Ghamdi MS (2002) A review of the pharmacy therapeutic effects of Nigella sativa. Pak. J. Med. Res., 41: 1-10.
10. Agrawal R, Kharya MD, Shrivastava R (1979) Antimicrobial and anthelmintic activities of the essential oil of Nigella sativa Linn. Indian. J. Exp. Biol., 17:1264-1265.
11. Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH (2003) The in vivo antifungal activity of the aqueous extract from Nigella sativa seed. Phytother. Res., 17:183-186.
12. Mahmoud MR, El-Abhar HS, Saleh S (2002) The effects of Nigella sativa oil against the liver damage induced by Schistosoma mansoniin mice. J. Ethnopharmacol., 79:1-11.
13. Besim H, Karayalcin K, Hamamci O, Güngör C, Korkmaz A (1998) Scolicidal agents in hydatid cyst surgery. HPB Surg., 10:347-351.
14. Hosseini SV, Ghanbarzadeh K, Barzin J, Sadjjadi SM, Tanideh N, Mehrabani D (2006) In vitro protoscolicidal effects of hypertonic glucose on protoscolices of hydatid cyst. Korean J. Parasitol., 44:239-242.
15. Adas G, Arikan S, Kemik O, Oner A, Sahip N, Karatepe O (2009) Use of albendazole sulfoxide, and combined solutions as scolicidal agents on hydatid cysts (in vitro study). World J. Gastroenterol., 15:112-116.
16. Chanda S, Baravalia Y, Kaneria M, Rakholiya K (2010) Fruit and vegetable peels - strong natural source of antimicrobics" In: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, FORMATEX Research Center, Badajoz, Spain,1: 444-450.
17. Spainhour CB (2005) Natural products. Drug Discovery Handbook, by Shayne Cox Gad Copyright © 2005 by John Wiley &Sons, Inc.
18. Atai Z, Ansari M, Torabi N (2007) Efficacy of olive leaf extract in the treatment of minor oral aphthous ulcers. Americ. J. Infect. Dis., 3(1):24-6.
19. Somova LI, Shode FO, Ramnanan P, Nadar A (2003) Antihypertensive, anti-atherosclerotic and antioxidant activity of triterpenoids isolated from Oleaeuropaea, subspecies Africana leaves. J Ethnopharmacol., 84: 299-305.
20. Benavente-Garcia O, Castillo J, Lorente J, Ortuno A, Del Rio JA (2000) Antioxidant activity of phenolics extracted from Oleaeuropea L. leaves. Food Chem., 68(4): 457-62.
21. Owen RW, Haubner R, Mier W, Giacosa A, Hull WE (2003) Food Chem. Toxicol., 41: 703-717.
22. Markin D, Duek L, Berdicevsky I (2003) In vitro antimicrobial activity of olive leaves. Myco., 46: 132-136.
23. Pereira AP, Ferreiara ICFR, Marcelino F, Valentao P, Andrade PB (2007) Phenolic compounds and antimicrobial activity of olive (Olea europaea L. Cv. Cobrancosa) leaves. Molecul., 12: 1153-1163.
24. Sudjana AN, D'Orazio C, Ryan V, Rasool N, Ng J (2009) Antimicrobial activity of commercial Olea europaea (olive) leaf extract. Int. J. Antimicrob. Agents, 33: 461-463.
25. Aytul KK (2010) Antimicrobial and antioxidant activities of olive leaf extract and its food applications. Turkey, MSc. Thesis, Graduate School of Engineering and Sciences of Izmir Institute of Technology.
26. Dahham SS, Ali MN, Tabassum H, Khan M (2010) Studies on Antibacterial and Antifungal Activity of Pomegranat (Punicagranatum L.). American-Eurasian J. Agric. & Environ. Sci., 9 (3): 273-281.
27. Kaur G, Jabbar Z, Athar M, Alam MS (2006) "Punicagranatum (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice," Food and Chem. Toxicol., 44(7):984-993.
28. Jurenka J (2008) "Therapeutic applications of pomegranate (Punica granatumL.): a review," Alternative Medicine Review, 13(2):128-144.
29. Bagri P, Ali M, Aeri V, Bhowmik M, Sultana S (2009) "Antidiabetic effect of Punica granatum flowers: effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes," Food Chem. Toxicol., 47(1): 50-54
30. Duman AD, Ozgen M, Dayisoylu KS, Erbil N, Durgac C (2009) "Antimicrobial activity of six pomegranates (PunicagranatumL.) Varieties and their relation to some of their pomological and phytonutrient characteristics," Molecules, 14 (5): 1808-1817.
31. Mahboubi A, Asgarpanah J, Sadaghiyani PN, Faizi M (2015) "Total phenolic and flavonoid content and antibacterial activity of Punicagranatum L. Var. pleniflora flowers (Golnar) against bacterial strains causing food borne diseases,"BMC Complementary and Alternative Medicine, 15(1: 366-372.
32. Gavanji S, Larki B, Bakhtari A (2014) The effect of extract of Punica granatumvar. pleniflora for treatment of minor recurrent aphthousstomatitis. Integ. Med. res., 3: 83-90.
33. Landa-Laracia JI, Alonso E, Gozalez-Uriate J, Rodriguez-Romano D (1997) Evaluation of scolicidal agents in experimental hydatid disease model.Eur.Sur.Res.,29:202-208.

34. Daniel WW (1988) Biostatistics. A foundation for analysis in Health Sciences, 4th ed., John Wiley & Sons, Inc.

35. Finney DJ (1977) Probit analysis, 3rd ed., London, Cambridge University Press,333.

36. Korukluoglu M, Sahan Y, Yigit A, Karakas R (2006) Antifungal activity of olive leaf (OleaEuropaea L.) extracts from the Trilye Region of Turkey. Ann. Microb., 56 (4): 359-362.

37. Lee OH, Lee BY (2010) Antioxidant and antimicrobial activities of individual and combined Phenolics in Oleaeuropea leaf extract. Bio Tech., 101: 3751-3754.

38. Zibaei M, Sarlak A, Delfan B, Ezatpour B, Azargoon A (2012) Scolicidal Effects of Oleaeuropaea and Satureja khuzestanica Extracts on Protoscolices of Hydatid Cysts. Korean J. Parasitol., 50(1): 53-56.

39. Gökmen M, Kara R, Akkaya L, Torlak E, Önen A (2014) Evaluation of antimicrobial activity in olive (Oleaeuropea) leaf extract. American J. Microbiol., 5 (2): 37-40.

40. Liu Y, McKeever LC, Malik N S A (2015) Assessment of the Antimicrobial Activity of Olive Leaf Extract against Foodborne Bacterial Pathogens. Frontiers in Microbiology| www.frontiersin.org. 8:1-8.

41. Pfaller MA, Messer SA, Boyken L, Hollis RJ, Rice C, Tendolkar S, Hollis RJ, Diekema DJ (2004) In vitro activities of voriconazole, posaconazole, and fluconazole against 4,169 clinical isolates of Candida spp. and C. neoformans collected during 2001-2002 in the ARTEMIS global antifungal surveillance program. Diagn. Microbiol. Infect. Dis., 48:201-205.

42. Ishida K, Mello JCP, Cortez DAG, Filho BPD, Ueda-Nakamura T, Nakamura CV (2006) Influence of tannins from Stryphnodendron adstringens on growth and virulence factors of Candida albicans. J. Antimicrob. Chemother., 58:942-949.

43. Hajifattahi F, Moravej-Salehi E, Taheri M, Mahboubi A, Kamalinejad M (2016) Antibacterial Effect of Hydroalcoholic Extract of Punica granatum Linn. Petal on Common Oral Microorganisms. Internat. J. Biomat., http://dx.doi.org/10.1155/2016/8098943.

