

Antibacterial Activity of Tannins Extracted from Some Medicinal Plants in vitro

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Abstract:

Objective: To investigate the inhibition effect of some condensed tannins on the growth of four type of bacteria in vitro.

Materials and methods: Susceptibilities were determined using diffusion method. Tests were conducted aerobically using AC agar medium.

Results: The result indicate that source and concentration are importance factors that influence antimicrobial activity of tannin.

Conclusion: Inhibition zones of growth were varied among plant seeds.

Key words: Bacteria, Tannins, Seed plants, Zone inhibition .

Introduction:

Tannins are astringent , bitter plants polyphenols that either bind and precipitate or shrink proteins. the term tannin refers to the use of tannin in tanning animal halides into leather, however the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecule. Tannins have molecular weights ranging from 500 to over 3000 ⁽¹⁾.

Nutrition and toxic effects of tannins present in various food stuffs, feed and fooder have been reviewed ^(2,3) In addition many biological activities and antibacterial promoting effect have been reported for plant tannin and flavor –oids and now they are investigated increasingly ^(4,5,6).

Tannins have traditionally been considered antinutritional but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. The new technologies used to analyze molecular and chemical structures have shown that a division into condensed and hydrolysable tannins is far too simplistic ⁽⁷⁾. Recent studies have demonstrated that products containing chestnut tannins included at low dosages (0.15-0.2 %) in the diet can improve broiler chicken performance and wellbeing ⁽⁸⁾. Studies on chestnut tannins have shown beneficial effects on silage quality in the round bale silages, in particular reducing ammonia and NPN (non protein nitrogen) in the lowest wilting level (9). Improved fermentability of soya meal nitrogen in the rumen has also been reported by Mathieu F and Jouany JP (1993) (10).

Studies by Gonzalez S. et al (2002)(11) on in vitro ammonia release and dry matter degradation of soybean meal comparing three different types of tannins (quebracho, acacia and chestnut) demonstrated that chestnut tannins are more efficient in protecting soybean meal from in vitro degradation by rumen bacteria.

Materials and methods:

Preparation of condensed tannin extracts: 10gms from (pimpinella anisum, punica granatum, nigella sativa) were dissolved in 100 ml distal water (DW), then we put the solutions were put in water bath (in 40 C) for 30 minutes, then tubes were centrifuged and took the supernatant .

We added 20ml lead tetra acetate 0.4% were added to the supernatant ,then we dried the samples and weighed ,then we calculated the percentage of tannins (as shown in table 1) then we prepared of tannins from water extract (1gm in 100ml D.W)for 10mg/ml,then we prepared sequence of concentrations (100,10,1,0.1,0.01,0.001) (mg/ml) (12).

Antibacterial activity of seed tannins:

Susceptibilities were determined using the disc diffusion method (National Mastitis Council, 1999). Discs (BD, sparks, MD)of 6mm in diameter saturated with each seed plants extracted solution were aseptically placed on agar media that had been spread with test bacteria(13,14). Tests were conducted aerobically using AC gar medium (all culture agar media; sigma; p.o Box14508, st., louis,Mo63(78USA) except for E.coli which was tested on Macconkey,s agar prepared by saturating with sterile water .

Result and Discussion :

Tannins compound in general has good activity against bacterial growth &that is because these compounds contain titanic acid against some genus of diseased bacteria through the ability of these compounds to dissolve the fatty layer of diseased bacterial wall that causes leakage of cell fluid out the cell &destroys it.

And has the ability to consist the hydrogen bond between OH group in phenol compound , that includes tanic acid ,nitrogen of amino acid in bacterial cell that lead to loss of this activity which leads to loss of vital action of cell & destroys it (15,16).

Table 1- The percentage of tannin in 3 seed plants

N	plants	Weight of plants (gm)	Weight of tannin(gm)	Percentage of tannin
1	<u>pimpinella anisum</u>	10gm	0.73	0.73%
2	<u>punica granatum</u>	10gm	0.7	0.7%
3	<u>nigella sativa</u>	10gm	0.4	0.4%

From table one could observe that, the highest percentage of tannin present in *pimpinella anisum* represents 0.73 % . followed by *punica*

granatum 0.7% and after that seeds of *nigella sativa* 0.4% this explains that the tannin percentage is different from one plant to other⁽¹⁷⁾.

Table 2- Effect the tannin extract (*pimpinella anisum*) against four types of bacteria

bacteria	inhibition Zone diameter(mm)					
	Concentration of tannins (mg/ml)					
	100	10	1	0.1	0.01	0.001
<i>E.P.E.coli</i>	14	11	7	0	0	0
<i>S. dysenteriae</i>	19	16	12	7	0	0
<i>Staph.aureus</i>	12	9	7	0	0	0
<i>k. pneumonia</i>	9	7	0	0	0	0

Note (discs 6mm in diameter saturated with each plant)

The effect of tannin which was extracted from these 3 plants on growth of 4 separated bacterial sample ,that is, *E.P. E.coli* , *s.dysenteriae* , *Staph.aureus* and *k. pneumonia* using different concentrations 0.001,0.01,0.1,1,10,100 mg\ml from tannin to each three types of plants in the study was explained in table (2).

Table 2 demonstrated , that tannin which was extracted from *pimpinella anisum* has more inhibited effect against 4 types of bacteria which were used in the research in different concentrations as explained in the figure (1) .

More inhibition was noticed when concentration was 100 mg \ ml *s.dysenteriae* & followed by *E.P.E.coli*.in(figure 1 represented this relation).

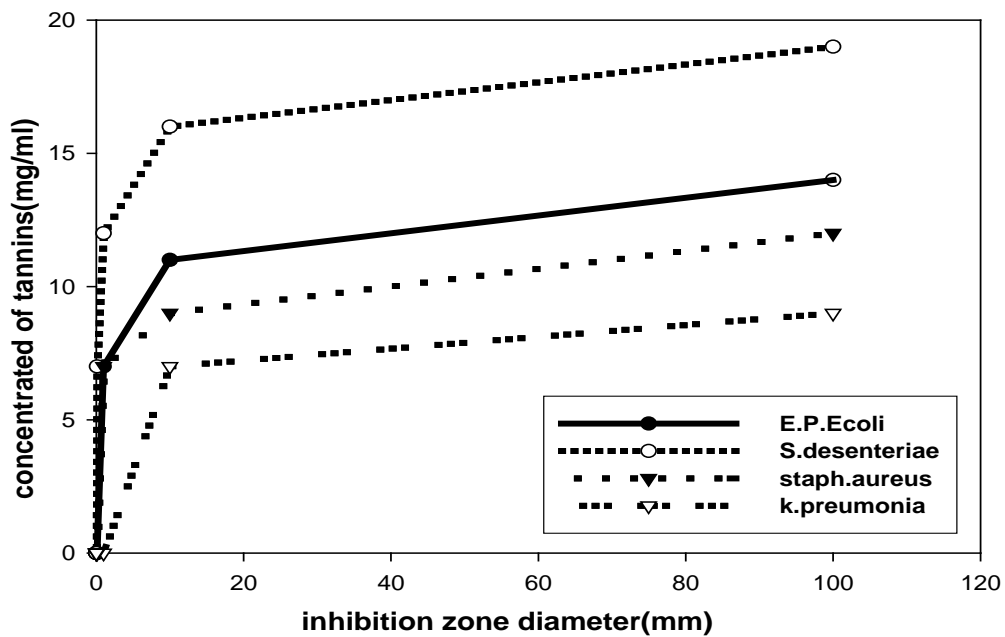


figure (1)

Table 3-Effect the tannin extract (*punica granatum*) against four types of bacteri

bacteria	inhibition Zone diameter(mm)					
	Concentration of tannins (mg/ml)					
	100	10	1	0.1	0.01	0.001
<i>E.P.E.coli</i>	11	9	7	0	0	0
<i>s.dysenteriae</i>	17	15	12	7	0	0
<i>Staph.aureus</i>	11	9	7	0	0	0
<i>k. pneumonia</i>	9	7	0	0	0	0

Table (3) explains more inhibition on 100 mg/ml concentration for *s .dysenteriae* that is clearly explained in figure(2).

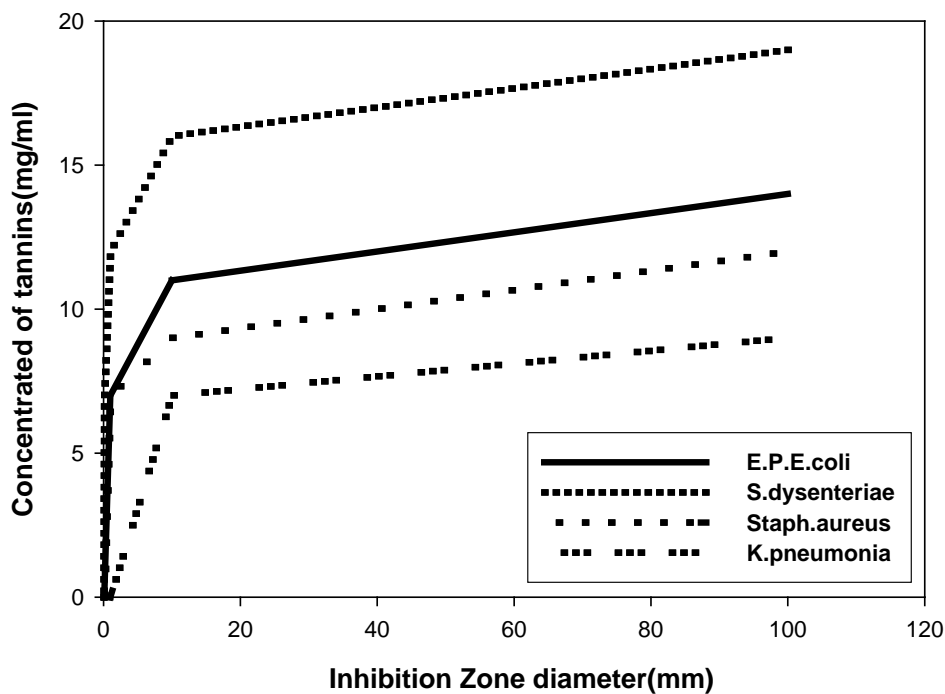


figure (2)

Table 4- Effect of tannin which was extracted from (*nigella sativa*)_ against four types of bacteria

bacteria	inhibition Zone diameter(mm)					
	Concentration of tannins (mg/ml)					
	1000	10	1	0.1	0.01	0.001
<i>E.P.E.coli</i>	9	0	0	0	0	0
<i>s.dysenteriae</i>	11	0	0	0	0	0
<i>Staph.aureus</i>	10	0	0	0	0	0
<i>k. pneumonia</i>	7	0	0	0	0	0

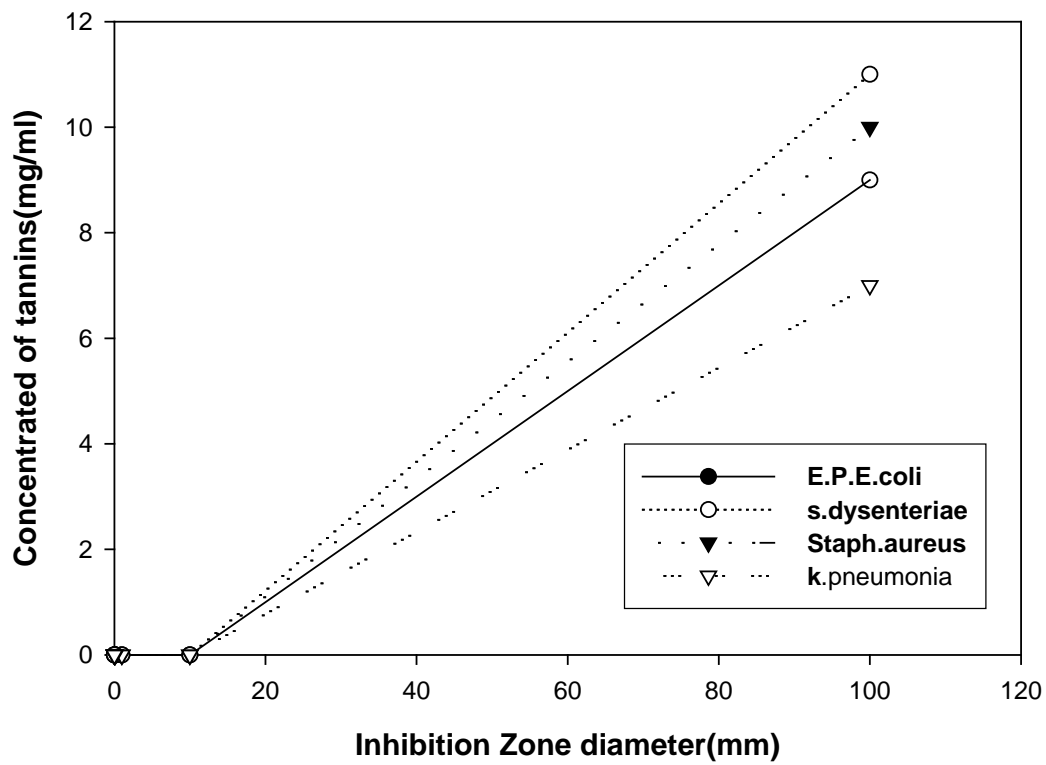


figure (3)

References:

- 1- Bate-Smith and Swain, 1962. Flavonoid compounds in comparative biochemistry Vol.3 75-809. Academic Press. New York.
- 2-Biomed, 2002. pharmacother, 56,276-82.
- 3-J.Agric,2003. food chem.. 51,3494-3501 R.corder, W.Mullen and A.Crozier.
- 4-Vattem.D.A.and R. Shetty , 2005. clinical nutrition 14(2) 120-130.
- 5-R.puupponen and K.M.O Ksman,(2001). OF applied microbiology 90(4) PP.494.
- 6-Wheeler and S.R. lewis, 2005. J Nutr biochemistry (16)360-367.
- 7-Muller-Harvey, I. and McAllan A.B. (1992). Tannins: Their biochemistry and nutritional properties. Adv. Plant Cell Biochem. and Biotechnol. 1: 151-217.

- 8-Schiavone, A., Guo, K., Tassone, S., Gasco, L., Hernandez, E., Denti, R. and Zoccarato, I. (2007) Effects of a Natural Extract of Chestnut Wood on Digestibility, performance Traits, and Nitrogen Balance of broiler Chicks. Poultry Science 87: 521–527.
- 9-Tobacco E., Borreani, G., Crovetto G. M., Galassi G., Colombo. D., and Cavallarin. L. (2002) Effect of Chestnut Tannin on Fermentation Quality, Proteolysis and Protein Rumen Degradability of Alfalfa Silage. J.Dairy Sci. 89: 4736–4746.
- 10-Mathieu F and Jouany JP (1993). Effect of chestnut tannin on the fermentability of soyabean meal nitrogen in the rumen. Ann Zootech 42, 127.

- 11-González, S., Pabón, M. L. and Carulla, J. (2002) Effects of tannins on in vitro ammonia release and dry matter degradation of soybean meal. Arch. Latinoam. Prod. Anim. 10(2): 97-101.
- 12- دلالي، باسل كامل والحكيم، صادق حسن، (١٩٨٧) تحليل الأغذية، دار الكتب، جامعة الموصل
- 13-Benson, H.J, 1998. Microbiological Application. 7th ed. WCB McGraw ,Hill, USA.P.51.
- 14-National Committee for Clinical Laboratory Standards (NCCLS) ,1993. Performance standards FOR antimicrobial disk susceptibility tests , NCCLS,Pennsylvania USA,M2-A5.
- 15-Aqeel,A,Kursheed,A.K.,and Sabiha,Q., (1989). Antibacterial activity of Julifra,PP.652-655.
- 16-AL-Azzawi, J.M.,AL-Shafi, N.M.,(1999). The effect of Gallic acid on human serum cholinestegase" ,Med. Earasite 22, PP 29-32.
- 17-BaglanH.M.A.(2007).Study the antibacterial activity of extracted tannins from some medicinal plants and their aqueous extracts. Diploma thesis .chemistry science .AL-Anbar university.