EFFECT OF ELLAGIC ACID ON SOME TYPES OF PATHOGENIC BACTERIA

Kadhem K. Ghudhaib[*], Ekbal R. Hanna [**] and Alaa Hussein Jawad[***]

[*] Department of Chemistry, College of Science for Women, Baghdad University, Iraq.
[**] Department of Biotechnology, College of Science, Baghdad University, Iraq.
[***] Department of Chemistry, College of Science, Al-Nahrain University, Iraq.

Abstract

Biological activity of ellagic acid toward some pathogenic strains bacteria (*Staphylococcus epidermatis, Bacillus cereus, Klebsiella pneumonia and Salmonella typhi*) was studied. The investigation was conducted using plate-agar method. Gentamycin and streptomycin were used as standard drugs. The results show that the biological activity of ellagic acid was more effective than gentamycin and streptomycin.Also, the minimum effective dose of ellagic acid was found to be 0.15 mg|ml, while the minimum effective doses of gentamycin and streptomycin were 0.3 mg|ml and 2 mg|ml, respectively.

Keywords: Antibacterial activity, Ellagic acid, Gram positive and gram negative bacteria.

Introduction

Ellagic acid is a phenolic acid present in many plant foods. The phenolic acids and flavonoids are forming a polyphenolic compounds that widely spread in plants, and they termed by a nutraceuticals [1]. Studies were referred to that polyphenols possess biological activities toward different diseases [2].

In more recently study, the activities of grape extracts against Helicobacter pyroly were studied, and found that the muscadine grape skin extract possessed the strongest activity compared with the grape seed and grape synergy extracts. The results may be attributed to the polyphenolic compounds that found in these extracts [3]. In another study polyphenolic compounds present in plant extracts have been tested for their activity against gram-positive and gram-negative bacteria of the genera Azotobacter, *Bacillus*, and *Pseudomonas* .The extracts offer promise as a source of raw material for isolation of polyphenolic compounds exhibiting bacteri-cidal activity [4].

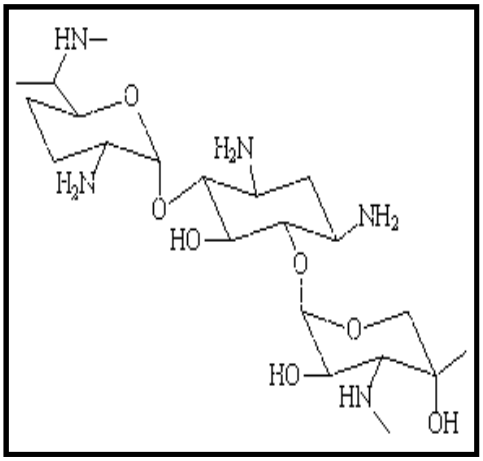
Dietary polyphenols are mostly derivatives or isomers of flavones, isoflavones, flavonols, catechins and phenolic acids and possess various biological properties. Research on the effects of dietary polyphenols on human health has developed strongly and supports a role for polyphenols in the prevention of degenerative diseases particularly cardiovascular disease, anti -inflammination, antiatherosclerosis, improve- ment of endothelial function as well inhibition of angiogenesis and cell proliferation activity[5].

The study of antimicrobial capacity of plant phenolics is well known [6, 7]. Crude extracts also showed good antimicrobial activity [8, 9].The walnut green husks aqueous of different cultivars were screened for their antimicrobial properties against *B.cereus, B.subtilis, S.aureus, E.coli, P.aeroginosa, Candida albicans* and *Candida neoformans.* The response for each microorganism was different and all the tested extracts revealed antimicrobial activity toward the studied microorganisms. The study was concluded that walnut green husks can be used as an easily accessible source of natural bioactive compounds against diseases [10].

Phytochemicals have been used as antibacterial, antidiarrheic, antidysenteric, antihepatoxic, antiviral, antigastric, fungicide, cyclooxygenase inhibitor and lipooxygenase inhibitor. These activities were attributed to their contents of ellagic acid, gallic acid, isoquercitrin, myricetin, quercitin and tannic acid [11]. The phytochemicals of thyme have been used as antibacterial and antifungal agents, and diuretic, urinary disinfectant and vermifuge [12]. Extracts of different polarities for medicinal plants were tested *in* preliminary

biological screening for their in *vitro* bactericidal activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeroginosa* and *Proteus vulgaris*. The results of this study were confirmed that there are promising extracts with high and broad antimicrobial activity [13]. G.S. Chakraborthy was reported that some extracts of medicinal plants were showed remarkable inhibitory action against various gram positive and gram negative bacteria and fungal species [14].

Ellagic acid is one constituent of many plant extracts [15, 16]. These extracts were reported to protect animal from Salmonella typhymurium. So it can be predicted that the regular intake of this type of extracts may be reduce the risk of getting typhoid fever. The study was referred to require of further work to explore the activity of this extract at molecular level against pathogen [17]. It was reported that some plant extracts could be a possible source to obtain new and effective herbal medicines to treat infection caused by multi drug resistant strains of microorganisms [18].

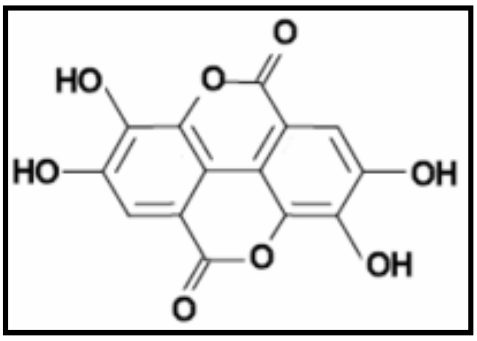


Gentamycin.

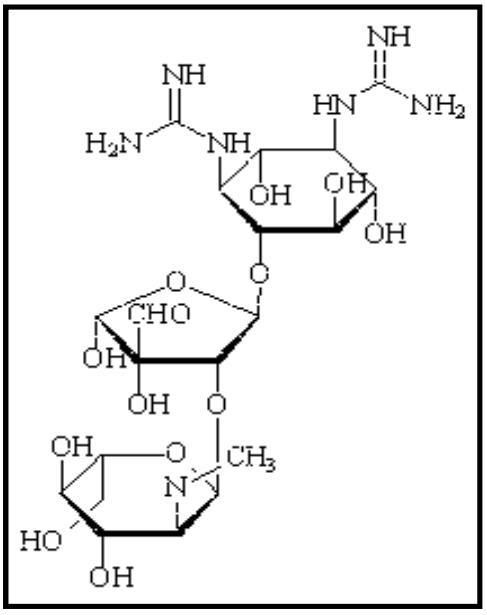
In another new study, researchers were suggested that ellagic acid, a compound found in plant products, may be effective against malaria and ultimately lead to new forms of treatment. Researchers performed further *in vitro* and *in vivo* study testing to clarify the levels of antimalaria properties of ellagic acid. The results of this study were appeared high activity in vitro against of plasmodium falciparum, one species of the parasite that causes malaria in human. Final findings by researchers suggest that ellagic acid may enhance the activity of current antimalaria drugs such as chloroquine, mefloquine and atovaquone [19].

The aim of this work is to evaluate the antibacterial activity of the prepared ellagic acid toward both gram-positive and gram-negative bacteria in comparison with the activity gentamycin and streptomycin.

Fig. (1) shows the structural formulae of ellagic acid, gentamycin and streptomycin.



Ellagic acid



Streptomycin

Fig. (1): The structural formulae of ellagic acid, gentamycin and streptomycin [1, 20].

Experimental

1. Preparation of ellagic acid:

Ellagic acid was prepared, purified and characterized as reported in literature [21]. Penta di m-galloyl β-D-glucose was used as starting materials for ellagic acid preparation.

2. Standard antibiotic drugs:

Streptomycin and gentamycin were of the highest available quality and purchase from Merck Company.

3. Microorganisms and culture conditions:

Staphylococcus epidermatis, Bcillus cereus (gram-positive), *Klebseilla pneumonia* and *Salmonella typhi* (gram- negative) bacteria were obtained from the biotechnology department, collage of science. The antimicrobial activity was achieved by plate agar method [22].Microorganisms were cultured aerobically at 37 C° for 24 hours in nutrient agar medium. The plates containing agar medium were inoculated by micro-organism suspensions, which are spreading on the surface. Each sample (100µl) was placed in a hole (3mm depth, 4 mm diameter) made in the agar layer. Under the same conditions, different solutions of gentamycin and streptomycin were used as standards. The diameter of inhibition zones were measured using a ruler with an accuracy of 0.5 mm.
A control using only inoculation was also carried out.

Results and Discussion

It was reported that the active concentrations of gentamycin and streptomycin are 0.3mg|ml and 2mg|ml, respectively [23]. Accordingly, six different concentrations of ellagic acid 4 mg|ml (C_1), 2 mg|ml (C_2), 1 mg|ml (C_3), 0.6 mg|ml (C_4), 0.3 mg|ml (C_5) and 0.15 mg|ml (C_6) were prepared to be used for the purpose of this study.

The Tables (1 to 4) show the results of the biological activity for ellagic acid and standard drugs, which were used in this study, against of *Staph. epidermatis, B.cereus, K. pneumonia and S. typhi.*

The results exhibit that ellagic acid is highly effective against all of the microorganisms under study.Compairing the effect of different concentrations of ellagic acid, gentamycin and streptomycin tells that no effect at concentration lowers than 0.3 mg|ml and 2 mg|ml for gentamycin and streptomycin respectively, whereas all the concentrations range of ellagic acid have been effective.

Table (1)
The biological activity of ellagic acid toward Staph. Epidermatis.

Sample no.	Comp.	C_1	C_2	C_3	C_4	C_5	C_6	Control
1	EA	20(mm)	18(mm)	12(mm)	10(mm)	8(mm)	7(mm)	-
2	GM	25	20	17	14	10	-	-
3	SM	20	15	-	-	-	-	-

**** EA = Ellagic acid, GM=Gentamycin, SM=streptomycin.***
**** (-) =Effective killing area is not significant.***
****C1-C6=Concentrations (4, 2, 1, 0.6 and 0.3 mg|ml).***
**** (mm) =inhibition zone diameter.***

Table (2)
The biological activity of ellagic acid toward B. cereus.

Sample no.	Comp.	C_1	C_2	C_3	C_4	C_5	C_6	Control
1	EA	19(mm)	17(mm)	13(mm)	11(mm)	10(mm)	8(mm)	-
2	GM	31	27	23	20	17		-
3	SM	24	18	-	-	-	-	-

Table (3)
The biological activity of ellagic acid toward K. pneumonia.

Sample no.	Comp.	C_1	C_2	C_3	C_4	C_5	C_6	Control
1	EA	19(mm)	15(mm)	13(mm)	11(mm)	10(mm)	9(mm)	-
2	GM	27	23	18	15	10	-	-
3	SM	26	20	-	-	-	-	-

Table (4)
The biological activity of ellagic acid toward S. typhi.

Sample no.	Comp.	C_1	C_2	C_3	C_4	C_5	C_6	Control
1	EA	17(mm)	15(mm)	13(mm)	11(mm)	10(mm)	8(mm)	-
2	GM	28	25	21	18	15	-	-
3	SM	22	17	-	-	-	-	-

Antibacterial activity of some plant extracts was interpreted on the basis of presence of some polyphenols such as quercitin and myricetin, which are enhancing their biological activity against pathogenic bacteria [24]. Suwipa et al were reported that the minimum inhibitory concentration of individual extracts of pomegranate rind and turmeric is recorded as the lowest concentration of drug [25].Many other studies were referred to that the activities of medicinal plant extracts can be explained by the presence of tannins, flavonoids,saponins and steroids[26,27].

Some information is available on oak nut gall as an effective medicinal plant against E.coli .This activity attributed to the presence of polyphenols, especially ellagic acid. The extract demonstrated inhibitory and bactericidal effects on all the tested strains with minimum inhibition concentrations at 0.78-1.56 mg|ml and minimal bactericidal concentrations at 1.56-3.12 mg|ml. However, antibacterial mechanism of this medicinal plant extract has not yet been elucidated [28].

As in tannin, ellagic acid may induce complexation with enzyme or substrate in bacteria cell. Ellagic acid toxicity may be related to its action on the microorganism membranes. Also, the ability of ellagic acid to form complexes with the essential metals in bacteria cell account for its toxicity [29].

Conclusion

High biological activity of ellagic acid was detected against all the tested microorganisms and at the lower concentrations in comparison with the standard drugs (streptomycin and gentamycin). It is evident from this study that ellagic acid could be utilized as a good antibacterial agent in pharmaceutical industry. Therefore; further work may be performed for its industrial and pharmaceutical application.

References

[1] F.Shahidi and M.Naczk. Phenolics in food and nutraceuticals.CRC Press, USA, 2003, pp.85.

[2] P.Assuntina, A. Manila, C. Massimiliano, A.Mauro, F. Evandro.and M. Anna. Effect of polyphenolic compounds on proteolytic activities of constitutive and immune proteasome .Antioxidant and redox signaling.Vol. 8,2006,121-129.

[3] C.Joseph, H. Guohui, H. Vivian, and J.Xiuping.Antibacterial effects of grape extracts on Helicobacter pyroly. Appl. Eniron.Microbiol.Vol.75, 2009, PP. 848-852.

[4] V.S.Nikitina and L.I. Kusmina. Anti - microbial activity of polyphenolic compounds isolated from plants of Geraniaceae and Rosaceae families. J Biochem. Microbiol.Vol. 43, 2007, PP. 705-712.

[5] S.Mukherjee, S. Das and D.M. Vasodvan. Role of polyphenols in diet and nutrition an updated review. Current Nutrition and Food Science.Vol. 5, 2009, PP.149-159.

[6] A.P.Pereira, I.R. Ferreira, F. Marcelino, P. Valento, F Andrade, R.Seabra, L. Estevinho, A. Bento and J.Pereira. Phenolic compounds and antimicrobial activity of olive leaves. Molecules. Vol. 12, 2007, PP.1153-1162.

[7] J.A.Pereira, I. Oliveria, A. Sousa, P.Valento, P. Andrade, I.R. Ferreira, F.Ferreres, A. Bento, R. Seabra and L.Estevinho.Walnut leave: phenolic compounds, antimicrobial activity and antioxidant potential of different cultivers.Food Chem. Toxicol. Vol.45, 2007, PP.2287-2295.

[8] J.A.Pereira, I. Oliveria, A. Sousa, I.R. Ferreira, A. Bento and L. Estevinho. Bioactive properties and chemical composition of six walnut cultivars. Food Chem. Toxicol.Vol. 46, 2008, PP. 2103-2111.

[9] A.Sousa,I.R. Ferreira,R.Calhela, P. Andrade,P. Valento,R. Seabra, L. Estevinho,A. Bento and J.A. Pereira. Phenolics and antimicrobial activity of

stoned table olive. Bioorg. Med. Chem. Vol. 14, 2006, PP. 8533-8538.

[10] I.Oliveria, A. Sousa, A. Bento, L. Estevinho. And J.A. Pereira. Total phenols, antioxidant potential and antimicrobial activity of walnut green husks. Food Chem.Toxicol.Vol.46, 2008, PP. 2326-2331.

[11] J.L.Rios .and M.C. Recio. Medicinal plants and antimicrobial activity. J Ethnopharmacol.Vol.100 2005, PP. 80-84.

[12] A.Ghalib, A, Assam, A. Kamel, A. Farah. Antibacterial effects of nutrace -utical plants growing in Palestine on pseudomonas aerogenosa.Turk. J Biol. Vol.30, 2006, PP.239-242.

[13] A.E. Awatif, M.O. Sara, E.O. Mohammed, and M.I. Khagali.In vitro antimicrobial activity on some Sudanese combretum species. Intern. Trop. Med. Vol. 2, 2007, PP.45-251.

[14] G.S.Chakraborthy. Antibacterial and antifungal studies of ekolania leaf extracts. Pharmacol.Vol. 1, 2009, PP. 393-397.

[15] H.G.Kim, J.H. Cho, E.Y. Jeong, J.H. Lim and S.H. Lee. Growth inhibiting activity of active components isolated from Terminalia chebula fruits against intestinal bacteria. J Food Prot.Vol. 69, 2006, PP.2205-2209.

[16] Juang LJ. And Sheu SJ.Chemical identification of the source of commercial fructus chebulae. Phyto- chem. Anal. Vol. 16, 2005, PP.246-251.

[17] KhanKH.Terminilia chebula reduces the oxidative stress induced by salmonella typhymurium and may reduce the risk of getting typhoid.Adv. Biol.Res.Vol.3, 2009, PP.1-8.

[18] Rosina K, Barira I, Mohd A, Shazi S, Anis A,Manazir A, Mashiatullah S, and Asad UK.Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules. Vol.14, 2009, PP. 586-597.

[19] S.Nijomang, B. Witcowski, D. Olagneir, M. Nicolau, S. Garci. And V.S.Benoit. In vitro and in vivo properties of ellagic acid in malaria treatment. Antimicro. Agents Chemother. 53, 2009, pp.1100-1106.

[20] N.S.Egorov.Antibiotics a scientific approach. Mir Publishers, Moscow, 1985, PP.245.

[21] Kadhem K. Ghudhaib, Ph.D., Baghdad University, Baghdad, Iraq. 2006.

[22] G.A.Washington.Laboratory proce- dures in clinical microbiology. Spring Verlog. New York.1981.

[23] J.Mary, A.Geaig, N.Mechael, E.George, W.H.David, T.S. Albert, M.S. Tana, C.T .Frend, T.T Raymond and P.W. Melvin. Performance standards for antibacterial suscep- tibility testing. west valley road, USA,2002,PP.72.

[24] A.Carolicka, E. Szpitter, E.Gilgenast, G. Romanic, M.Kaminski and E. Loikows. Stimulation of antibacterial naphtha-quinones and flavonoids accumulation in carnivorous plants grown in vitro by addition of elicitors. Enz Microb. Tech.42, 2008, PP.216-221.

[25] U.Suwipa, S.Tanomjit, S.Pichnoi, S. SupreedeeR.Pranee and A.Ithrat. Study on antioxidant antimicrobial activities of turmeric clear liquid soap for wound treatment of HIV patients.J. Sci. Tech. 27, 2005, pp.569-578.

[26] A.B.Aliyo,A.M.Musa,M.S.Abdullahi and A.O.Oyewale.Phytochemicals and antimicrobial properties ludwigia suffraticosa.Inter Jor Pure Appl Scs. 2, 2008, PP.1-5.

[27] E.Babpour,S.Abdulhamid Angajiand S.Mahdi Angaji.Antimicrobial effects of four medicinal plants on dental plaque.J Mid Plant Res.3, 2009, PP. 132-137

[28] Sakol S and Spayang PV. Morphological and structural changes in cell structure of enterohaemorrhagic E.coli following treatment with nut galls.J Electr Micros.58, 2009, PP.315-320.

[29] A.Misanori, F.Kosuyasu, Y.Osumu, O. Takashi and I. Kiji. Antibacterial action of several tannins against staphylococcus aureus. J. Antimicrob. Chemother.48, 2001, PP. 487- 491.

الخلاصة

يتضمن هذا البحث دراسة الفعالية الحياتيـــة لحـــامض الالاجيك تجاه بعض عزلات البكتريـــا المرضـــية (بكتريـــا المكورات العنقوديه والعصويه والكلبسله الرئويه والسلمونله التيفوئيه). اجريت الدراسة بطريقة اطباق بتري. تم استعمال الجنتامابيسين والستريبتومايسين كعقارين قياســيين. بينـــت نتائج البحث الى ان الفعالية الحياتية للحامض كانت اكبر من الجنتامايسين والستربتومايسين.كذلك وجد ان اقـــل جرعـــة مؤثرة لحامض الالاجيك هي 0.15 ملغم | مل بينما كانـــت اقل جرعة مؤثرة للجنتامايـــسين والستريبتومايـــسين هـــي 0.3 ملغم | مل و 2 ملغم | مل على التوالي.