International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 5 (May-2015) pp. 43-48

www.ijcrar.com

Combination of antioxidant and ampiclox do not offer effective antimicrobial activity against *Staphylococcus aureus*

Batool Hazim Al-Daherie*

Department of Microbiology, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq

Corresponding author

KEYWORDS

Gallic acid, Ampiclox; Antimicrobial; *Staphylococcus aureus*

A B S T R A C T

Gallic acid is related to phenolic compounds that exert multi-biological effects in vitro and in vivo studies. Its effect against methicillin resistant *Staphylococcus* aureus has been reported. This study aims to evaluate the effect of synthetic gallic acid against Co-ampiclox resistant *Staphylococcus aureus.* This study was done in the Department of Microbiology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq from October 2014 to December 2014. A total number of thirty *Staphylococcus aureus* isolated from infected wounds and burns were obtained from the laboratories of Al-Yarmouk Teaching Hospital. The antibacterial effect of Co-ampiclox (25µg/ml), gallic acid at serial concentrations (1-64µg/ml) alone or in combination was examined in vitro using broth dilution technique. Four out of thirty isolates were resistant to 25 µg/ml ampiclox. Gallic acid *per se* at low concentration (1µg/ml) inhibits the growth of the susceptible isolates by 18.4% while at higher concentrations failed to exert any antimicrobial effect and reduced the antimicrobial effects of Co-ampiclox against the susceptible *Staphylococcus aureus.* Gallic acid at low concentration (1µg/ml) inhibits the growth of the resistant isolates by 13.7% while its combination with Co-ampiclox at concentration 2µg/ml inhibits the mean growth of bacteria by 18.9%. Synthetic gallic acid exerts a direct inconsistent antibacterial effect against Co-ampiclox susceptible and resistant *Staphylococcus aureus.* Any combination of gallic acid and other antibacterial agents should be used with caution.

Introduction

Gallic acid is a 3,4,5-trihydroxybenzoic acid related to phenolic compounds found in plants (Shahrzad *et al.*, 2001). It is commonly used in the pharmaceutical industry and in particular, as a standard for determining the phenolcontent of various analyses. It is a powerful antioxidant that helps to prevent oxidative damage (Aruoma *et al.*, 1993). Studies have shown that gallic acid is effective in inhibiting neuronal death and in preventing cellular mutations and to be toxic to cancer cells (Paolini *et al.*, 2015).

It inhibits histamine release and the expression of pro-inflammatory cytokines; therefore, it is useful in management of asthma and other allergic conditions (Kim *et al.,* 2006). The antibacterial effect of gallic acid was demonstrated against *H. pylori* in vitro by an agar-well diffusion method and by scoring colony forming units and its effect is dependent on the concentration and contact time (Díaz-Gómez *et al.,* 2013). The antibacterial effects of gallic acid against gram positive and negative are attributed to irreversible changes in membrane properties by inducing hydrophobic changes, decrease in negative surface charge, and occurrence of local rupture or pore formation in cell membranes with consequent leakage of essential intracellular constituents (Borges *et al.,* 2013). *Staphylococcus aureus* isolated from burns and infected wounds, showed resistant to gentamicin, ciprofloxacin, norfloxacin, rifampicin, chloramphenicol and ampiclox (Ojo *et al.,* 2014). Most studies carried on the effects of the antioxidants against resistant *Staphylococcus aureus* used the extracts of herbs or medicinal plants rather than using the synthetic antioxidants (Mekinić *et al.,* 2014; Malviya *et al.,* 2014). Essential oils obtained from garlic, Chinese chive and onion all of which had antioxidant properties, were used against various micro-organisms including *Staphylococcus aureus.* However, these essential oils contained a number of antioxidants rather that one specific antioxidant (Mnayer *et al.,* 2014). This study aims to evaluate the effect of synthetic gallic acid against ampiclox resistant *Staphylococcus aureus.*

Materials and Methods

This study was undertaken in the Department of Microbiology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq from October 2014 to December 2014. The study was approved by the Institutional Scientific Committee. A total number of thirty *Staphylococcus aureus* isolated from infected wounds and burns were obtained from the laboratories of Al-Yarmouk Teaching Hospital. The swabs obtained from the patients were aerobically cultured on different media including blood agar, and mannitol salt agar. They were incubated at 37ºC for 24 hours. The isolates were diagnosed according to well-known established microbiological methods that are principally based on morphological characteristics, Gram stain method and conventional biochemical testing (Forbes *et al.,* 2007). Preparation of bacterial suspension was achieved with a sterile wire loop. The top surface of 3–5 isolated colonies of the *Staphylococcus aureus* to be tested were picked from the original culture and introduced into a test tube containing 10 ml of sterile Muller Hinton broth and the turbidity was compared and adjusted with the turbidity standard using McFarland tubes as prescribed by Vandepitte *et al.* (1991).

Experimental design

Both ampiclox (vial, 500 mg) and gallic acid (powder) were purchased from local markets. They were dissolved in distilled water and different concentrations were prepared in the appropriated volume (25 µl for each concentration) to be suitable for the volume of the microtitre plates. The cut-off level of ampiclox concentration that discriminated the susceptible and resistant isolate is 25µg/ml.

To each well of plane microplate, a final volume of 200 µl of broth suspended with bacterial growth was added in one series. In the other series, the following were added:

- distilled water (served as a control)
- or Co-ampiclox (at a 25 µg/ml concentration)

- or gallic acid (at a serial concentrations ranged from 1 to 64 µg/ml)
- or a combination of Co-ampiclox and gallic acid.

The minimum inhibitory concentrations of Co-ampiclox and gallic acid were determined using the reader of the Enzyme Linked Immuno-Sorbent Assay by measuring the absorbance at 630 nm, taking into consideration the absorbance of bacterial growth in absence or presence of distilled water or gallic acid, ampiclox or the combination of gallic acid and Co-ampiclox. The results are expressed as number, percentage, mean and median.

Results and Discussion

Four isolates out of thirty showed resistance to co-ampi-cloxacillin (25µg/ml). The percentage of inhibition of susceptible isolates ranged between 7.2 to 77.1% (Table 1). Gallic acid *per se* at low concentration (1µg/ml) inhibits the growth of the susceptible isolates by 18.4% while higher concentrations failed to exert any antimicrobial effect (Figure 1). Figure 1 shows that gallic acid supplementation to the Co-ampiclox reduced the antimicrobial effects of Co-ampiclox against the susceptible *Staphylococcus aureus*. Combination of Co-ampiclox and gallic acid at concentration 32µg/ml inhibit the growth of bacteria by 13.2% while the median inhibition of Co-ampiclox alone is 50.9% (Table 1). Gallic acid *per se* at low concentration (1µg/ml) inhibits the growth of the resistant isolates by 13.7% while higher concentrations failed to exert any antimicrobial effect (Figure 2). Combination of Co-ampiclox and gallic acid at concentration 2µg/ml inhibit the mean growth of bacteria by 18.9% (Figure 2). There is a variation in the percentage of growth inhibition in respect to the isolate (Table 2).

The results of this study show that the antibacterial effect of gallic acid is inconsistent and does not follow concentration-effect pattern. In addition, gallic acid antagonizes the effect of Co-ampiclox against Co-ampiclox susceptible isolate, while its effect against resistant isolates is better when combined with Co-ampiclox. The possible explanation of this result may be attributed to the direct effect of gallic acid against the microorganism rather than to its effect as that mentioned together with antibacterial. Gallic acid does not induce any harmful effect on the normal cell but it can induce necrosis in abnormal cells. Hsieh *et al.* (2014) reported that gallic acid induced hepatic stellate cellular death but not hepatocyte death via a rapid burst of reactive oxygen species leading to increased intracellular calcium. The other explanation of gallic acid toxicity is related to the inhibition of protein synthesis via inhibiting certain enzymes related to the metalloproteinase enzymes (Kuo *et al.*, 2014). Literature reviews do not reveal the effect of gallic acid on bacterial ribosomes. Therefore, its effect against bacteria is not related to the inhibition of bacterial protein synthesis. Recently, Luis *et al.* (2014) reported that the mechanisms of antibacterial effect of gallic acid against *Staphylococcus aureus* are by inhibiting bacterial adhesion and the production of α-hemolysin. There is no interference with bacterial cell membrane. Moreover, Lee *et al.* (2014) reported that synergistic bacterial effect against methicillin resistant *Staphylococcus aureus* was observed when gallic acid is grafted with chitosans and combined with β-lactams. Further study also reported the synergism between gallic acid and several antibacterial agents (excluding penicillin and their derivatives) of different mechanisms against *Ps. aeruginosa* without elucidating the mechanism of action (Jayaraman *et al.*, 2010).

Table.1 The percent inhibition of the bacterial growth achieved by 25 µg/ml Co-ampiclox

Isolate No.	Inhibition (%)
1	16.4
2	42.0
3	40.1
4	42.8
5	51.1
6	50.6
7	35.7
8	19.1
9	52.2
10	7.2
11	71.8
12	70.0
13	77.1
14	59.3
15	67.6
16	49.2
17	44.0
18	34.6
19	10.7
20	76.4
21	40.9
22	44.7
23	74.8
24	43.5
25	44.5
26	42.9

Table.2 Percentage of growth inhibition in the presence of gallic acid supplementation to Co-ampiclox

Isolate No.	Gallic acid concentration (µg/ml)	Inhibition (%)
1	16	22.5
2	2	10.8
3	2	36.0
4	1	9.5

Figure.1 Effect of gallic acid at different concentration on Co-ampiclox-susceptible *Staphylococcus aureus*

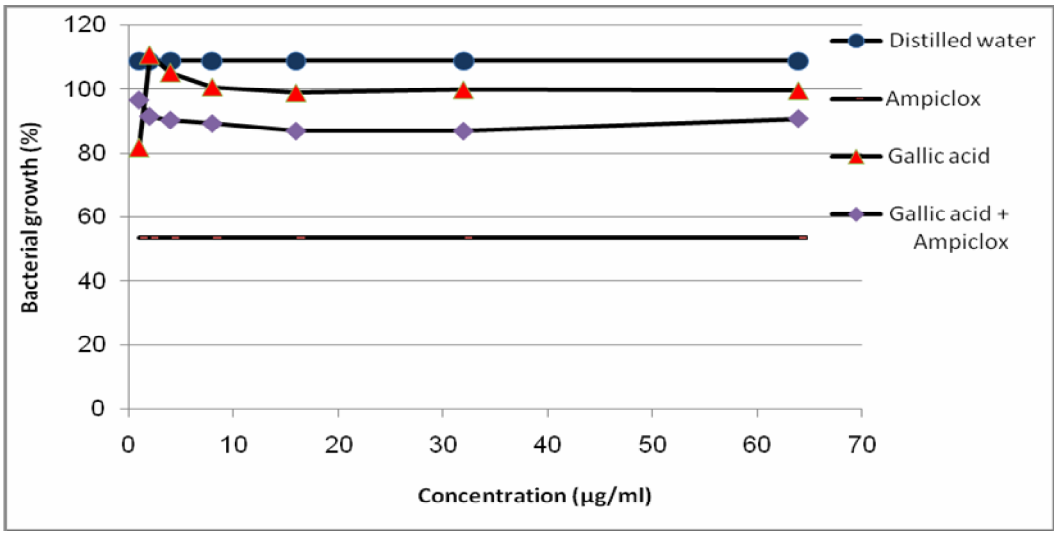
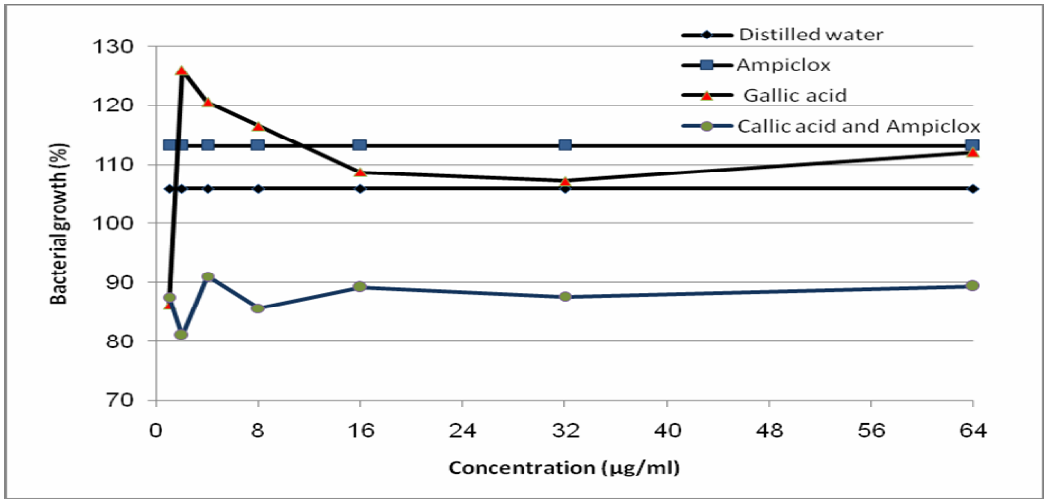


Figure.2 Effect of gallic acid at different concentration on Co-ampiclox-resistant *Staphylococcus aureus*



Conclusion

Synthetic gallic acid exerts a direct inconsistent antibacterial effect against ampiclox susceptible and resistant *Staphylococcus aureus.* Any combination of gallic acid and other antibacterial agents should be used with caution.

References

Aruoma, O.I., Murcia, A., Butler, J., Halliwell, B. 1993. Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *J. Agric. Food Chem.*, 41(11): 1880–1885.

Borges, A., Ferreira, C., Saavedra, M.J., Simões, M. 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.*, 19(4): 256–265.

Díaz-Gómez, R., López-Solís, R., Obreque-Slier, E., Toledo-Araya, H. 2013. Comparative antibacterial effect of gallic acid and catechin against

Helicobacter pylori. *LWT - Food Sci. Technol.*, 54(2): 331–335.

Forbes, B.A., Sahm, D.F., Weissfeld, A.S. 2007. Bailey and Scotts "Dignostic Microbiology", 12th edn. Mosby Elsevier Press, St. Louis, Missouri.

Hsieh, S.C., Wu, C.H., Wu, C.C., Yen, J.H., Liu, M.C., Hsueh, C.M., *et al.* 2014. 'Gallic acid selectively induces the necrosis of activated hepatic stellate cells via a calcium-dependent calpain I activation pathway. *Life Sci.*, 102(1): 55–64.

Jayaraman, P., Sakharkar, MK., Lim, C.S., Tang, T.H., Sakharkar, K.R. 2010. Activity and interactions of antibiotic and phytochemical combinations against Pseudomonas aeruginosa in vitro. *Int. J. Biol. Sci.*, 6(6): 556–68.

Kim, S.H., Jun, C.D., Suk, K., Choi, B.J., Lim, H., Park, S., Lee, S.H., Shin, H.Y., Kim, D.K., Shin, T.Y. 2006. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. *Toxicol. Sci.*, 91(1): 123–31.

Kuo, C.L., Lai, K.C., Ma, Y.S., Weng, S.W., Lin, J.P., Chung, J.G. 2014. Gallic acid inhibits migration and invasion of SCC-4 human oral cancer cells through actions of NF-κB, Ras and matrix metalloproteinase-2 and-9. *Oncol. Rep.*, 32(1): 355–361.

Lee, D.S., Eom, S.H., Kim, Y.M., Kim, H.S., Yim, M.J., Lee, S.H., *et al.* 2014. Antibacterial and synergic effects of gallic acid-grafted-chitosan with β-lactams against methicillin-resistant *Staphylococcus aureus* (MRSA). *Can. J. Microbiol.*, 60(10): 629–638.

Luís, Â., Silva, F., Sousa, S., Duarte, A.P., Domingues, F. 2014. Antistaphylococcal and biofilm inhibitory activities of gallic, caffeic, and chlorogenic acids. *Biofouling*, 30(1): 69–79.

Malviya, S., Arvind, A.Jha., Hettiarachchy, N. 2014. Antioxidant and antibacterial potential of pomegranate peel extracts. *J. Food Sci. Technol.*, 51(12): 4132–4137.

Mekinić, I.G., Skroza, D., Ljubenkov, I., Krstulović, L., Možina, S.S., Katalinić, V. 2014. Phenolic acids profile, antioxidant and antibacterial activity of chamomile, common yarrow and immortelle (Asteraceae). *Nat. Prod. Commun.*, 9(12): 1745174–8.

Mnayer, D., Fabiano-Tixier, A.S., Petitcolas, E., Hamieh, T., Nehme, N., Ferrant, C., *et al.* 2014. Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae family. *Molecules*, 19(12): 20034–20053.

Ojo, S.K., Sargi, B.O., Esumeh, F.I. 2014. Plasmid curing analysis of antibiotic resistance in beta-lactamase producing Staphylococci from wounds and burns patients. *Pak. J. Biol. Sci.*, 17(1): 130–133.

Paolini, A., Curti, V., Pasi, F., Mazzini, G., Nano, R., Capelli, E. 2015. Gallic acid exerts a protective or an anti-proliferative effect on glioma T98G cells via dose-dependent epigenetic regulation mediated by miRNAs. *Int. J. Oncol.*, 46(4): 1491–1497.

Shahrzad, S., Aoyagi, K., Winter, A., Koyama, A., Bitsch, I. 2001. 'Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. *J. Nutr.*, 131(4): 1207–1210.

Vandepitte, J., Engback, K., Piot, P., Heuck, C.C. 1991. Basic laboratory procedures in clinical bacteriology. J.P. 76 Pp.