



Antibacterial Activity of Phenolics compounds against Pathogenic Bacteria

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ABSTRACT

The increased resistance of pathogenic microorganisms is ascribed to the extreme use of antibiotics. To contend with onset of resistant microbes, scientists have been seeking in finding of new antimicrobials. Plant secondary metabolites attract the attention of scientist towards plants, synthesizing numerous secondary metabolites, which are known to be involved in defense mechanisms. In the last few years it is recognized that some of these molecules also have health beneficial effects, including antimicrobial properties. The antimicrobial screening of natural secondary metabolites (phenolics compounds) like Gallic acid, Quercetin, Caffeic acid, Coumaric acid, Tannic acid and Catechol were carried out against microbial species (*E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*). Gallic acid was not effective in inhibiting the growth of *Bacillus subtilis* but *E. coli* and *P. aeruginosa* were sensitive. Quercetin, Caffeic acid, Coumaric acid, Tannic acid and Catechol were effective in inhibiting the growth of the organisms.

Keywords: Phenolics compounds, antibacterial activity, MIC.

INTRODUCTION

In recent years much attention has been devoted to natural antioxidant and their association with health benefits owing to increase in pathogenic resistance as the antimicrobial agents are losing their efficacy.^{1,2} Overuse of antibiotics is making the bacteria resistant. The three fundamental mechanisms of antimicrobial resistance are enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets and changes in membrane permeability to antibiotics. Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. Therefore, there is need of new search in the area of antimicrobial agents that may not induce bacteria to develop resistance as antibiotics do. After prolong dependence on synthetic or chemical agents, present age once again has more inclination towards natural medicines. It is a well established fact that plants produces many chemicals to protect themselves from insects, bacteria and fungus, such plant products can be exploited as elementary sources of pharmaceutical products.²⁻⁵ Estimated species of plants on Earth are 250,000 to 500,000 and very small percentages (1 to 10%) of these are at present used as foods⁶. It is possible that even more can be used for medicinal purposes.⁷ Phenolic products constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites. Secondary plant metabolites have received great attention in recent decades due to their presumed role in the cancer prevention, as atherosclerosis preventing agents for cardiovascular diseases, and in the slowdown of the aging process.^{8,9} In addition, they show other beneficial biological properties, such as antimicrobial and antioxidant activities.¹⁰⁻¹⁴ The goal of present study was to determine whether the etiologic

agent is resistant or sensitive to the natural antimicrobial agents being tested.

MATERIALS AND METHODS

Preparation of Phenolic Compounds

Phenolic compounds (Gallic acid, Quercetin, Caffeic acid, Coumaric acid, Tannic acid and Catechol) were obtained from Himedia Pvt. Ltd. Those compounds were dissolved in methanol and stored at 4°C.

Microorganisms

The antibacterial activity of phenolic compounds against referenced three bacteria was evaluated: *Escherichia coli* (Serotype O59) isolated from Calf, *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424)

Antimicrobial Test

The antibacterial test was performed by following agar disc diffusion method.^{15,16} Bacterial strains were grown on Luria Broth media (LB) for 18 to 24 h at 37°C. The inoculums of the indicated bacterial strains were transferred into physiological suspension medium and adjusted to 0.5 Mac Farland turbidity standards.¹⁷ A sterile filter disc impregnated with 10 µl of each extract suspended in methanol was placed on the infusion agar seeded with bacteria. Then, Petri dishes were kept at 37°C for 24 h. Chloramphenicol standard discs were used as positive antibiotic controls. Discs impregnated with 10 µl of pure methanol were used as negative controls. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. After incubation the inhibition zones were measured to an accuracy of 0.1 mm. All experiment was carried out in triplicate. The antibacterial activity was expressed as the mean of inhibition diameter zone produced.



Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of extract were also determined by Micro dilution Broth method.

The minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$ was determined by comparing the different concentration. The inoculation and reading procedures allow simultaneous testing of several antimicrobial agents against organisms, also the results of testing may be

determined by spectrophotometry.¹⁸ The least concentration of the samples with no growth was taken as the MIC.

RESULTS AND DISCUSSION

In the present study, we focused on antibacterial activity of pure phenolic compounds (Himedia Pvt. Ltd.) The antibacterial activity of gallic acid and quercetin was tested against pathogenic bacteria and compared with chloramphenicol (Table1).

Table 1: Antibacterial activity of phenolics compounds at 250 μg (n=3)

Phenolics compounds	<i>Escherichia coli</i> (Serotype O59)	<i>Pseudomonas aeruginosa</i> (MTCC 424)	<i>Bacillus subtilis</i> (MTCC 441)
Gallic Acid	++	++	-
Quercetin	++	++	+
Caffeic acid	++	++	++
Coumaric acid	++	++	+
Tannic acid	++	++	+
Catechol	++	++	+
Chloramphenicol	+++	+++	+++

-: no antimicrobial activity

+: Moderate antimicrobial activity, i.z. of sample 3–7 mm+ inhibition zone of methanol

++: Clear antimicrobial activity, i.z. of sample 8–10 mm. + inhibition zone of methanol

+++ : Strong antimicrobial activity, i.z. of sample. i.z. >10 mm. + inhibition zone of methanol

Table 2: Minimum Inhibitory Concentration (MIC) of phenolics compounds in ppm after 24 hour of incubation for Gram positive & Gram negative bacteria

Phenolics compounds	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
Gallic Acid	<20	<60	NA
Quercetin	<60	<80	<60
Caffeic acid	<20	<60	<60
Coumaric acid	<40	<40	<80
Tannic acid	<20	<40	<80
Catechol	<80	<40	<60

Antimicrobial activity of commercial phenolics compounds like Gallic Acid, Quercetin, Caffeic acid, Coumarin, Tannic acid and Catechol was investigated against microorganism. The results revealed that different bacterial species exhibit different antimicrobial sensitivities towards the tested phenolic compounds. Generally, the Gram-negative bacteria (*Escherichia* and *Pseudomonas*) were observed to have more antimicrobial susceptibility than Gram-positive (*Bacillus*). The results also illustrated that Gallic acid is not effective against *Bacillus subtilis* but *E. coli* and *P. aeruginosa* were sensitive. Other compounds also showed the moderate activity against *Bacillus subtilis*. Aksun and others (2009a) reported that Gallic acid was reported to be highly antimicrobial against Gram-negative pathogens.¹⁹ Antibacterial activities of Quercetin, Caffeic acid, Coumaric acid, Tannic acid and Catechol also showed the moderate activity against *Bacillus subtilis* and clear antimicrobial activities against *E. coli* and *P. aeruginosa*. R. Puupponen-Pimia and others (2001) reported that

phenolic extracts of eight berries inhibited the growth of selected Gram-negative bacteria and were not active against Gram-positive.²⁰ Treated samples of pathogenic bacteria using relevant MICs for each, were incubated for 24 hours at appropriate incubation temperature and conditions. The range of MIC was 20 $\mu\text{g/ml}$ to <80 $\mu\text{g/ml}$ as shown in Table 2. Caffeic acid, naringenin, p-coumaric acid and syringic acid have antimicrobial activity towards Gram-positive like *Bacillus spp.*, and Gram-negative bacteria including *E. coli*.^{19,21,22} The results of this study confirm these studies having similar antimicrobial activities.

CONCLUSION

Frequent and adequate use of antibiotics attributes the resistance to microorganism. Plants secondary metabolites seek the attention of the scientist as they have ability to check the growth of microorganism. In Present study, the commercial phenolics compounds have taken to test their antimicrobial activity. In this



study, it was found that all the phenolics compounds had antimicrobial activity except gallic acid. Gallic acid was not effective against *Bacillus subtilis*.

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