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Rutin : A Potential Antibiotic Resistance Modifying Agent

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ABSTRACT

In vitro resistant profiling study of 11 antibiotics (ciprofloxacin, tetracycline, trimethoprim, ampicillin, chloramphenicol, norfloxacin, erythromycin, nitrofurantoin, cefixime, amikacin and lincomycin) was done against five gram-positive and three gram-negative bacterial strains. All strains were found multidrug resistant. *Pseudomonas aeruginosa* was found to be Extended Spectrum β Lactamase (ESBL) producer. Ten phytochemicals (gallic acid, jasmonic acid, piperine, quercetin, rutin, salicyclic acid, shikimic acid, berberine and tannic acid) were evaluated for antibacterial activity. Only rutin demonstrated the potential antibacterial activity. But in the synergistic studies, all 10 phytochemicals with various antibiotics revealed that gallic acid, salicyclic acid, berberin, rutin and quercetin complimented strong inhibitory potential with chloramphenicol, tetracycline and erythromycin. The study validated potential of phytochemicals as genuine source of antibiotic resistance modifying agents.

Key words : Phytochemical, antibiotics, ESBL, bacteria, antibiotic resistant

INTRODUCTION

The emergence variant strain of COVID-19 Corona virus is the talk of globe at the moment. All around media, debates have started about mutation variants, antibiotics, anti-viral drugs resistance of microbes at all levels. But the rapid emergence of antibiotics resistance has been occurring, endangering the efficacy of antibiotics since decades. The antibiotics resistance crisis has been attributed to the overuse and misuse of these medications, as well as lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Shin *et al.*, 2018). Antibiotic resistance does not mean the body is becoming resistant to antibiotics; it is that bacteria have become resistant to the antibiotics designed to kill them. It has the potential to affect people at any stage of life, affects the veterinary and agriculture industries. It is one of the world's most urgent public health problems (Gonzalez-Bello *et al.*, 2019). The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928. Antibiotics were first prescribed to treat serious infections in the 1940s. Penicillin was successful in controlling bacterial infections among World War II soldiers. However, shortly thereafter, penicillin resistance became a substantial clinical problem, so that, by the 1950s, many of the advances of the prior decade were threatened (Spellberg and Gilbert, 2014). In response, new beta-lactam antibiotics were discovered developed and deployed, restoring confidence. However, the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was identified during that same decade, in the United Kingdom in 1962 and in the United States in 1968. Theories of natural selection lead to antibiotics resistance as antibiotics remove the sensitive competitors, leaving resistant bacteria behind to reproduce. This has strongly altered the micro biome of living beings as well as environment. The widespread inappropriate use of antibiotics emerged as one of the main causes of antibiotic resistance, which call for the development of antibiotic stewardship

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programs and global surveillance networks (Giacomini *et al.*, 2021).

Herbal drugs and phytochemicals have been used for their effective antimicrobial activity from ancient times and there is an increasing trend for development of plant based natural products for the prevention and treatment of pathogenic diseases (Ayaz *et al.*, 2019). One of the strategies for effective resistance modification is the use of antimicrobial agent-phytochemical combinations that will neutralize the resistance mechanism, enabling the drug to be effective against resistant microbes (Haroun and Al-Kayali, 2016). These phytochemicals can work by several strategies, such as inhibition of target modifying and drug degrading enzymes or as efflux pumps inhibitors. A plethora of herbal extracts, essential oils and isolated pure compounds have been reported to act synergistically with existing antibiotics, antifungals and chemotherapeutics and augment the activity of these drugs (Hubsch *et al.*, 2014).

MATERIALS AND METHODS

Bacterial strains were procured from Microbial Type Culture Collection (MTCC), Institute of Microbiology Technology (IMTECH), Chandigarh and National Dairy Research Institute (NDRI), Karnal and screened for the prevalence of antibiotic resistant and Extended Spectrum β Lactamase (ESBL) production. All bacterial cultures were maintained in Nutrient Agar slants. Five gram-positive (*Staphylococcus aureus* : MTCC3160, *Staphylococcus epidermidis* : MTCC3086, *Staphylococcus hominis* : MTCC4435, *Bacillus cereus* : MTCC430, *Bacillus subtilis* : MTCC121) and three gram-negative (*Escherichia coli* : MTCC1885, *Klebsiella pneumonia* : MTCC4030 and *Pseudomonas aeruginosa* : MTCC7453) bacterial strains were maintained.

High potency discs of 11 antibiotics belonging to different classes purchased from Hi-media Laboratories, Mumbai were : Antibiotics ciprofloxacin (5 µg), tetracycline (30 µg), trimethoprim (5 µg), ampicillin (10 µg), chloramphenicol (30 µg), norfloxacin (10 µg), erythromycin (15 µg), nitrofurantoin (300 µg), cefixime (5 µg), amikacin (30 µg) and lincomycin (2 µg). To detect the Extended Spectrum beta Lactamase (ESBL) production in gram-negative bacterial strains, the antibiotic discs of amoxicillin-clavulanate (30 cg) and 3rd generation cepalosporins (3 GC) cefotaxime (30 µg), ceftriaxone (30 µg), cefpodoxime (10 µg) and 4th generation cephalosporins (4 GC) cefepime (30 µg) were also purchased form Hi-media laboratories.

Antibiotic susceptibility test was determined by the disk diffusion method as described by the Clinical and Laboratory Standards Institute.

Susceptibility of gram-negative bacteria strains was determined to a range of extended spectrum cephaloporins such as cefpodoxime, cefotaxime, ceftriaxone in accordance with CLSI susceptibility guidelines. ESBL production was further confirmed by modified Double Disc Synergy Test. The discs of 3 GC and 4 GC were placed 15 and 20 mm apart, respectively, centre to centre to that of the amoxicillin-clavulanate. The distortion in zone was considered positive ESBL production.

Ten phytochemicals : Gallic acid, jasmonic acid, piperine, plumbagin, quercetin, rutin, salicyclic acid, shikimic acid, berberine and tannic acid were purchased from Sigma Aldrich.

The antibacterial activity of phytochemicals was done by Agar well diffusion method. 100 µl of the inoculum of tested organism (1.5×10^6 CFU/ml) was poured into semi-hot nutrient agar plates and allowed to solidify. The seeded plates were bored 8 mm to create wells for loading testing phytochemical samples. Sterile DMSO was negative control and ciprofloxacin as positive control. The zone of inhibition was measured.

Minimum Inhibitory Concentration (MIC) of phytochemicals was determined by micro dilution technique using 96 well micro-titre plates as described by the National Committee for Clinical Laboratories Standards. The reading of tubes was measured as a function of turbidity at 660 nm. The tests were conducted in triplicates.

RESULTS AND DISCUSSION

In the study, all the bacterial strains were found to be resistant against any three or more

antibiotics. All strains were susceptible to norfloxacin, ciprofloxacin and amikacin. Two strains (*Bacillus* sp.) exhibited resistance to erythromycin and six strains to trimethoprim except *S. hominis* and *P. aeruginosa*. All strains showed 100% resistance to ampicillin, chloramphenicol, nitrofurantoin, cefixime, tetracycline and lincomycin. Gram-positive bacteria exhibited resistance to antibiotics as compared to gram-negative. The bacterial strains were found to be more resistant to protein synthesis inhibitor antibiotics as compared to nucleic acid inhibitor antibiotics. The studies supported work where *P. aeruginosa* exhibited resistance to various β-lactam antibiotics and non β-lactam antibiotics (Gonzalez-Bello *et al.*, 2019). Mex efflux proteins mediating multidrug resistance were also identified in *P. aeruginosa* (Tafti *et al.*, 2020). Another study reported 66.6% strains of *S. aureus, P. aeruginosa* and *E. coli* resistant to tetracycline (Mosafa *et al.*, 2014). Out of three gram-negative bacterial strains studied for ESBL producers, only *P. aeruginosa* was found positively confirmed. Our results are in agreement with earlier reports which also reported ESBL production in *P. aeruginosa* (Gonzalez-Bello *et al.*, 2019). However, there were some reports which detailed on the β lactamase production in *E. coli* also, which was contradictory to the present study (Nepal *et al.*, 2017). Differences among the results could be related to the use of strains with different resistance profiles and different MTCC numbers. Among the 10 phytochemicals studied only the rutin showed antibacterial activities against five bacterial strains with zone of inhibition ranging from 10 -13 mm. The MIC for rutin ranged from 30 to 15 µl/ml. The compound showed activity against *S. epidermitis, S. hominis, B. subtilis, K. pneumoniae* and *P. aeruginosa*. In synergistic studies, the best interaction was shown by gallic acid, salicyclic acid, berberin, rutin and quercetin with antibiotics chloramphenicol, tetracycline and erythromycin. The best synergism was observed for tetracyclin with gallic acid followed by rutin and quercetin. There were different efficacies against different bacterial strains. Erythromycin showed good synergism (20 mm ZOI) against *Staphylococcus* strains, as well as *E. coli* (15 mm ZOI), *K. pneumoniae* (23 mm) and *P. aeruginosa* (25 mm). Nucleic acid synthesis inhibitors ciprofloxacin and norfloxacin did not show synergistic effect.

These findings have potential implications in delaying the development of resistance as the antibacterial effect is achieved with lower concentrations of both drugs (antibiotics and phytochemicals). Synergistic interaction between two agents, in which one agent enhances the effect of the other and together they act more efficiently than as individual agents, motivated many scientists to examine and assess the significance of synergistic acting plant derived compounds and traditional antibiotics. It is well known that plant extracts possess antibacterial properties but also, the ability to enhance the activity of an antibiotic in combination with it. That ability of plant active substances reflects in modification or blocking of resistance mechanism so that bacterium becomes sensitive to antibiotic or the antibiotics acts when in lower concentrations. Such an approach, besides reducing the effective dose of antibiotics on one side, also reduces the side effects of antibiotics as medicine on the other side.

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