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RESEARCH ARTICLE

Antibacterial Activity of Combination of *Polyalthia longifolia* Thw. Extract, Cow Urine Distillate and Streptomycin

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ABSTRACT:
Bioenhancers are substances which promote or augment the bioactivity or bioavailability or the uptake of drugs in combination therapy. The present study was undertaken to determine antibacterial potential of a combination that contained pericarp extract of *Polyalthia longifolia* Thw. (Annonaceae), Cow urine distillate (CUD) and Streptomycin. The antibacterial activity of pericarp extract, CUD, Streptomycin and the combination was determined against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* by Agar well diffusion method. Extract, CUD and antibiotic have shown higher inhibition of Gram positive bacteria. Combination resulted in higher inhibition of test bacteria when compared to antibiotic. Among bacteria, *S. aureus* was inhibited to more extent followed by *P. aeruginosa* and others. The combination could be useful in the inhibition of the pathogens as the dosage of antibiotics in the combination is much lesser than the dosage when used alone. The reduction in the antibiotic dosage could result in lesser side effects and also the cost of antibiotic could be reduced. The combination tested in this study could be effective against antibiotic resistant bacteria.

KEYWORDS: *Polyalthia longifolia*, Cow urine distillate, Agar well diffusion, Streptomycin, Bioenhancers

INTRODUCTION:
Bacterial infections are responsible for highest percentage of infections and are the major cause of disease throughout the world. The discovery and use of antibiotics has led to the suppression of diseases caused by variety of pathogens particularly bacteria. Antibiotics have distinct effect on pathogenic microbes. They could interfere with cell wall synthesis, increase bacterial membrane permeability; inhibit bacterial protein synthesis, etc. However, bacteria have been able to evolve to become resistant to antibiotics. The emergence of multidrug resistant bacteria became the major cause of failure of treatment. Several factors influence the increase in antibiotic resistance namely the microbial characteristics, selective pressure of antibiotic use of transmission of resistant organisms and resistant traits from resistant strains to susceptible ones [1-3].

It is now standard clinical practice to use combination of two or more antibiotics as a result of development of resistance in organisms.

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The combination usually comprises antibiotics that have different mode of actions in order to prevent resistance development and to improve therapy. This approach is very useful in expanding the antimicrobial spectrum, to prevent emergence of mutants, minimize toxicity and to obtain synergistic activity [2,4]. Even though the combined antibiotic therapy to treat diseases is advantageous, it will not be effective for a long period of time because of possible changes in the susceptibility pattern of bacteria. Therefore, the development of new classes of antimicrobial is of significant importance. Plants have tremendous medicinal importance to man. The medicinal properties of plants are due to the characteristic secondary metabolites such as phenols, flavonoids, alkaloids, etc contained in them. These metabolites have several bioactivities such as antimicrobial, antioxidant, antihelmintic, anticancer activities etc. The plant derived compounds become the base for the development of drug and may be used for the treatment of diseases. The plant derived medicines are safer, have profound therapeutic benefits and have more affordable treatments. There are no or minimum side effects as compared to synthetic drugs and usually have effectiveness beyond the symptomatic treatment of diseases [2,5].

Bioenhancers are substances which promote or augment the bioactivity or bioavailability or the uptake of drugs in combination therapy. Such bioenhancers have been reported from plants. Studies have revealed that the combination of plant extracts with antibiotics could reduce the concentration of antibiotics required for inhibition of pathogenic microbes [6,7]. It is now proved that cow urine and its distillate also possess bioenhancing effect on antimicrobial activity of antibiotics and plant extracts [8,11].

Polyalthia longifolia Thw belongs to the family *Annonaceae* and is a native of Sri Lanka. It is grown in gardens throughout the warmer parts of India. It is known as Mast tree, Fake Asoka tree, False Devadaru and Cemetry tree in English and Devadaari in Ayurveda. It is having febrifuge action and causes cardiac depression. The stem bark contains clerodane diterpenes, polyalthialdoic acid and kolavenic acid. The stem and its bark also contain the cytotoxic aporphine alkaloid, liriodenine, besides *nor*-oliveroline and oliveroline-beta-N-oxide. Azafluorene alkaloids are also present in the bark and leaves. The leaf exhibits fungitoxic activity [12]. Combined antibacterial activity of plant extracts, cow urine distillate and antibiotics has not been documented earlier. In this study, we have determined inhibitory efficacy of a combination that comprises of methanol extract of pericarp of *Polyalthia longifolia* Thw. (Annonaceae), Cow urine distillate and Streptomycin.

MATERIALS AND METHODS:

Collection and identification of fruits of *P. longifolia:*

The mature fruits were collected from the college campus and authenticated by Prof. Rudrappa D, Dept. of Botany, SRNMN College of Applied Sciences, Shivamogga-01, Karnataka. Voucher specimen (SRNMN/RAMP/Pl-2011-12) was deposited in the department herbaria for future reference.

Extraction:

The collected fruits were washed thoroughly to remove extraneous matter on surface, pericarp was separated, shade dried, powdered mechanically and subjected for extraction. A known quantity of powdered pericarp (100gm) was subjected to soxhlation and exhaustively extracted with methanol (HiMedia, Mumbai). The extract was filtered, concentrated in vaccum under reduced pressure and dried in the desiccator [13]. Methanolic extract was subjected to preliminary phytochemical screening to screen secondary metabolites namely alkaloids, saponins, flavonoids, glycosides, tannins, steroids and terpenoids [14].

Preparation of Extract, Antibiotic and their combination:

Extract was prepared in dilute Dimethyl sulfoxide (DMSO) to get a concentration of 50mg/ml of DMSO. Streptomycin (1mg/ml of sterile distilled water) was used as standard antibiotic. CUD was purchased from local shop and used directly. Combination of extract, antibiotic and CUD was prepared by mixing 1ml of each in a test tube.

Antibacterial activity of extract, antibiotic, cow urine distillate and their combination:

In this study, two Gram negative bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* and two Gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis* were tested for their susceptibility. Agar well diffusion method was performed to determine antibacterial activity. Test tubes containing sterile nutrient broth were aseptically inoculated with the pure cultures of test bacteria and incubated at 37°C for 24 hours. The broth cultures of bacteria were inoculated by swabbing sterile nutrient agar plates followed by punching of wells of 6mm diameter in agar. The extract, CUD, Control (10% DMSO), Streptomycin and extract-CUD-Streptomycin combination were transferred into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded [15].

RESULTS:

Phytoconstituents namely tannins, alkaloids, flavonoids, glycosides and sterols were detected in the methanol extract while terpenoids and saponins were not detected.

Table 1: Antibacterial activity of extract (E), CUD, Streptomycin (S) and their combination

Test bacteria	Zone of inhibition in mm			
	E	S	CUD	E+S+CUD
B. subtilis	25	34	22	36
S. aureus	28	37	22	38
P. aeruginosa	25	33	20	37
E. coli	22	31	19	36

The result of inhibitory activity is shown in Table 1. The extract was found to cause marked inhibition of bacteria tested. Among bacteria, Gram positive bacteria have shown more sensitivity to extract when compared to Gram negative bacteria. *E. coli* was inhibited to lesser extent when compared to other bacteria. Inhibition caused by standard antibiotic was higher than that of methanol extract. Here also, inhibition of Gram positive bacteria was higher when compared to Gram negative bacteria. CUD also caused more inhibition of Gram positive bacteria. DMSO did not cause any inhibition of bacteria. In case of combination of extract, CUD and antibiotic, marked inhibition of the bacteria was observed and the inhibition recorded was higher than that of inhibition caused by antibiotic alone. Among bacteria, *S. aureus* was inhibited to more extent followed by *P. aeruginosa* and others.

DISCUSSION:

The antibacterial activity of combination of pericarp extract, CUD, antibiotic and their combination was tested against two Gram positive and two Gram negative bacteria. Results were recorded as presence or absence zones of inhibition around the well. The presence of inhibitory zone around the well was taken as positive for antibacterial activity. Marked inhibition was observed in case of Gram positive bacteria by extract, CUD and antibiotic when compared to Gram negative bacteria. Inhibition caused by the combination was higher when compared to inhibition caused by individual treatments.

Antimicrobial activities of tannins, flavonoids, saponins, terpenoids, alkaloids, steroids and glycosides have been well documented [16-22]. The extract of the selected plant in this study was found to possess most of the phytoconstituents. The antibacterial activity of extract in this study could be chiefly due to the presence of these phytoconstituents.

Antibiotics resistance has become a hurdle, which has increased substantially in recent years, is posing an ever increasing problem. *Staphylococcus aureus* and *Pseudomonas aeuroginosa* have been recognized as most common bacteria which have developed resistance against several antibiotics. *P. aeruginosa* is a major hospital borne pathogen which is particularly dangerous to patients and population having weak resistance. Nearly 50% of *S. aureus* strains (MRSA) have developed resistance to antibiotic Methicillin**.** For most MRSA strains, glycopeptide-type drugs such as vancomycin are the only effective antimicrobial agents. However, vancomycin-resistant *S. aureus* (VRSA) has been reported. This is because of increasing and indiscriminate use of antibiotics [3, 23-25].

Plants produce multidrug resistance inhibitors that enhance the activity of antibiotics. Several studies have revealed the reduction in MIC values of Antibiotics when combined with crude extracts or purified phytochemicals. The sub inhibitory levels of *Catha edulis* were found to reduce the MIC of tetracycline and penicillin against oral pathogens [6]**.** Polyphenols have been reported to reverse β-lactam resistance in MRSA[7]**.** Phytochemicals have been reported to have resistance modulating abilities on various antibiotics against resistant strains of *S. aureus* [26,27]**.** The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment [28].

In India, Cow is worshipped as Kamadhenu and drinking cow urine is practiced since several years. Cow urine distillate is transparent liquid obtained by distilling cow urine and is being in use for several diseases and disorders. It has greater acceptance and is being used even to treat cancer. It has been found that cow urine and cow urine distillate also possess bioenhancing role which helps in reducing the dose and cost of antibiotics. CUD has been identified as more effective bioenhancer than cow urine [8]**.** A few studies have been carried out on bioenhancing role of CUD on antibacterial activity of plant extracts. Enhanced activity of fruit extract of *Capsicum frutescens* on combining with CUD was observed in a study by Rakshita *et al,*[9]**.** Surabhi *et al.* [10] showed increased antibacterial activity of combination of pericarp extract of *Polyalthia longifolia* and antibiotic. Recently, Swathi *et al.* [11] showed bioenhancing role of CUD on antibacterial activity of a macrolichen *Everniastrum cirrhatum.*

CONCLUSION:

In this study, the combination of pericarp extract, antibiotic and CUD resulted in inhibition of both Gram positive and Gram negative bacteria. The combination resulted in higher inhibition when compared to inhibition caused by antibiotic alone. Thus, the combination could be useful in the inhibition of the pathogens. Also, the dosage of antibiotics in the combination is much lesser than the dosage when used alone. The reduction in the antibiotic dosage could result in lesser side effects and also the cost of antibiotic could be reduced. Majority of the test bacteria used in this study have already developed resistance to a wide variety of antibiotics. The combination tested in this study could be effective against drug resistant bacteria. The inhibitions observed in this study are the effects resulting from the combination of antibiotic, extract and CUD and it has not been documented earlier. Further, toxicity studies and *in vivo* studies are needed to confirm these findings.

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