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SYNERGISTIC EFFECT ASSESSMENT OF PUNICA GRANATUM PERICARP EXTRACT AND BETA LACTUM ANTIBIOTICS AGAINST MRSA STRAINS

[1]T.Durai Anand,[2]S.Muthuramkumar,[3]V.Ganesan

[1]Department of Microbiology, V. H. N. S. N. College (Autonomous), Virudhunagar
[2] Department of Botany, V. H. N. S. N. College (Autonomous), Virudhunagar

ABSTRACT:

Punica granatum is an extremely significant medicinal plant with commercial significance and is known for its antioxidant potential. The pericarp is a leftover unwanted part of the Punica granatum fruit that has been reported to have several medicinal uses in traditional medicine. This study focuses on analyzing the antibacterial potential of the pericarp extracts and its synergistic antibiotic effect with some beta lactum antibiotics against *methicillin- resistant Staphylococcus aureus(MRSA).* The antibacterial actions of water extract of dried out Punica granatum pericarps were tested against MRSA strains by agar well diffusion method. The pericarp extract sterilized by bacterial filter proved advanced inhibitory outcome against the MRSA strains. Then the pericarp extract is combined with beta lactum antibiotics such as cephalothin, cloxacillin, ofloxacin and cefoxitin. Cephalothin and ofloxacin are effective against MRSA strains individually and shows synergistic effect with Punica granatum fruit pericarp extracts. But cloxacillin and cefoxitin, are not effective against MRSA strains individually but shows synergistic effect when combined with fruit pericarp extracts. The synergistic effect of antibiotic is assessed using Checkerboard Assay method.

Index Terms – Punica granatum, Beta lactum antibiotics, *Methicillin- Resistant Staphylococcus aureus,* Medicinal Plant

I. INTRODUCTION

Millions of people suffer severe infections every year due to drug-resistant staphylococcus bacteria. This kind of staph bacteria is identified as methicillin-resistant *Staphylococcus aureus* (MRSA), even though it's resistant to widespread antibiotics, including penicillin and amoxicillin. MRSA infections initially appeared regularly in hospitals and nursing homes. MRSA infections can turn into life-threatening if the bacterium spreads from the skin, to the lungs, the bloodstream, or other organs in the body. A number of diseases caused by MRSA bacterial strains are not curable and fatal because of their high resistance rate towards the clinical antibiotics. (Nikaido 2009).More than seventy percentage of bacterium reported have acquired antibiotic resistance. (Harvey et al. 2015).Traditionally, the medicinal plants and their extracts are used to treat various infectious diseases in India. The several medicinal plants and their extracts have been recorded as sources of antimicrobial agents (Rachana et al. 2012). The antibacterial potential of natural plants has a great focus in discovery of new drugs around the world (Rios and Recio 2005). The medicinal plants combined with pharmaceutical drugs have convinced herb-to-drug relations and the probable effects of these interactions comprise synergistic increase of the antibacterial potential and decrease of poor side effects of synthetic antibiotics. (Borchers et al. 1997). The herb-to-drug interaction approach may well direct to the discovery of new antibiotics and the reuse of synthetic antibiotics towards which bacteria have produced resistance (Saklani and Kutty 2008).In the present study the Punica granatum fruit pericarp extract is combined with some beta lactum antibiotics and their synergistic antibiotic is assessed.

II. MATERIALS AND METHODS:

SAMPLE COLLECTION AND PREPARATION

The fruit pericarps of Punica granatum were collected in fresh condition from the nearby villages of Virudhunagar. The collected peels were processed in Microbiology Research Laboratory of VHNSN College (Autonomous), Virudhunagar. The Collected peel samples were washed with distilled water and impurities were cleaned off. The cleaned peels were air dried for two weeks and constantly monitored to avoid fungal contamination during this process. The completely dried pericarps were ground to fine powder and stored in a sterilized air tight container at the room temperature for further analysis.

PREPARATION OF AQUEOUS EXTRACT

The powdered pericarps material of 1.5gm was mixed in 50 mL of double distilled water and incubated in shaking incubator at 25^0C with continuous shaking at 150 RPM for 7 days. By using Whatman filter paper, it was filtered and filtrate was kept at 40^0C to evaporate all the solvent so that only crude aqueous extract only left. The filtered crude aqueous extract was mixed with in equal volume (Debnath*et al*., 2014).

SEPARATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) STRAINS FROM WOUNDS OF PATIENTS

The pus samples from wound were used to isolate MRSA strains. The specimens of pus were collected from Bose Clinical Lab, Madurai. For MRSA strains enrichment, the Fluid thioglycollate medium was used. The specimen was inoculated on blood agar plates. The plates were incubated aerobically at 37° C overnight.

KIRBY-BAUER DISK DIFFUSION SUSCEPTIBILITY TEST

The Kirby-Bauer disk diffusion test was developed for this study with the sterile disks loaded with the MRSA strains and antibiotics cephalothin, cloxacillin, ofloxacin and cefoxitin were injected on the disk. The sensitivity patterns of the test organism were recorded for each synthetic antibiotics.(Acar et al 1991). The Himedia sterile disks were used for this study. The sensitivity patterns of test organism were recorded again for MRSA strains reaction with fruit pericarps extract.

DETERMINATION OF SYNERGY BY CHECKERBOARD ASSAY

The checkerboard assay technique was used to assess the antibacterial combinations *in vitro*. The synergism between the combined Punica granatum and the various antibiotics which was studied by the Kirby- Bauer method was further confirmed by the checker board assay. A final volume of 1ml in each tube was used. 0.5 ml of broth containing antimicrobials (0.25 ml of broth for each drug if two drugs are being tested) and 0.5 ml of broth containing a suspension of the organism to be tested. Since the final volume was 4 times great as the volume of broth for each antimicrobial with this method, the antimicrobial concentration used in the initial solutions is 4 times greater than the desired final concentration. The results were interpreted by the friction inhibitory index FIC_{index}

$$FIC_{index} = FIC_A + FIC_B$$

FIC_A is the friction inhibitory index of drug A and FIC_B is the friction inhibitory index of drug B. If the value of FIC_A+FIC_B is less than 0.5, there is synergy between two antimicrobial agents.

III. RESULTS AND DISCUSSION

In the modern years, for the healthcare needs, the medicinal plants with preventive and therapeutic effects are contributing more. [Holetz et al 2002). Many pharmacological studies have confirmed that many of medicinal plants are known to acquire antimicrobial properties. The side effects associated with the over prescription of traditional synthetic antibiotics and time to time resistant microorganisms development against antibiotics are ever-increasing (Meléndez et al 2006, Nazet. Al 2007). Among the medicinal plants, Punica granatum has an important role in traditional medicine. Pomegranate is known as a rich source of pharmacological properties which have been evaluated due to antiparasitic, antibacterial, antifungal, and anti-cancer effects as well as protection against herpes virus, and decrease in atheromatous plaque formation and reduction of systolic blood pressure (Reddy et al 2007, Kim et al 2002) . In the present study, the synergistic effect of Punica granatum pericarps aqueous extracts with synthetic antibiotics were studied for development of new drug against MRSA.

The antibiotic sensitivity pattern of isolated *Staphylococcus aureus*strains against antibiotics such as cephalothin, cloxacillin, ofloxacin and cefoxitin were studied by Kirby - Bauer disk diffusion test. The results were noted in Table I.

Table I : Antibiotic sensitivity pattern of isolated strains of *Staphylococcus aureus*for synthetic antibiotics

S.No.	Antibiotics	Concentration (µg)	Average Diameter of zone of inhibition (mm)	Reaction of the organism
1.	Cephalothin	20	10	Intermediate sensitive
2.	Ofloxacin	20	15	Sensitive
3.	Cloxacillin	20	1	Resistive
4.	Cefoxitin	20	0	Resistive

With the application of fruit pericarps aqueous extract on the disk with MRSA, the sensitivity pattern was recorded. The results were recorded in Table II.

Table II: Antibiotic sensitivity pattern of isolated strains of *Staphylococcus aureus* for Punica granatum pericarps extract

S.No.	Organism	Concentration (µg)	Diameter of zone of inhibition (mm)	Reaction of the organism
1.	48%	15	21	Sensitive
2.	37%	20	22	Sensitive
3.	15%	25	26	Sensitive

Each synthetic antibiotic is combined with equal volume of Punica granatum pericarps aqueous extracts and the sensitivity patterns were recorded for each combination. The results were tabulated as in table III.

Table III: Antibiotic sensitivity pattern of isolated strains of *Staphylococcus aureus* for Punica granatum pericarps extract and synthetic antibiotics

S.No.	Antibiotics	Concentration (µg)	Average Diameter of zone of inhibition (mm)
1.	Cephalothin + Punica granatum pericarps extract	15 µg+15 µg	32
2.	Ofloxacin +Punica granatum pericarps extract	15 µg+15 µg	36
3.	Cloxacillin + Punica granatum pericarps extract	15 µg+15 µg	29
4.	Cefoxitin + Punica granatum pericarps extract	15 µg+15 µg	30

To assure the synergistic effect of synthetic antibiotics with Punica granatum pericarps extract, the checker board assay technique was done. The friction inhibitory index FIC_{index} was found out for each combinations and they were recorded. From the table IV, it was interpreted that for all the combinations the FIC_{index} were less than 0.5 and it proved the synergetic effect of Punica granatum pericarps extract against synthetic antibiotics.

Table: IV. Assessment of synergy between combined Punica granatum pericarps extract and synthetic antibiotics against MRSA strains by checkerboard assay

Sl. No	Antibiotics	FIC_A+FIC_B Value	Interpretation
1.	Cephalothin + Punica granatum pericarps extract	0.25	Presence of synergy
2.	Ofloxacin + Punica granatum pericarps extract	0.26	Presence of synergy
3.	Cloxacillin + Punica granatum pericarps extract	0.312	Presence of synergy
4.	Cefoxitin + Punica granatum pericarps extract	0.312	Presence of synergy

From the results, it is well interpreted that the aqueous extract of Punica granatum pericarps has antibiotic potential against MRSA strains. When pericarps extract is combined with synthetic antibiotics, it shows synergic antibiotic effect against MRSA for each combinations.

IV. CONCLUSION

The most important conclusion of our study is the synergistic effect of the combination of plant extract of Punica granatum with some ß-lactam antibiotic such as cephalothin, cloxacillin, ofloxacin and cefoxitin against MRSA strains. The most important finding of our study is that the skin peelings of Punica granatum are found to have strong antibacterial activity against MRSA strains. The antibiotics cephalothin and ofloxacin are effective against MRSA strains individually and shows synergistic effect with combined plant extracts. The antibiotics cloxacillin and cefoxitin, are not effective against MRSA strains individually but shows synergistic effect with combined Punica granatum extracts. Punica granatum aqueous extract has antibiotic potential against MRSA and it gets enhanced when it is combined with Beta lactum antibiotics.

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