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Synergy of Plant-Derived Extracts with Antibiotics Against *Staphylococcus aureus* USA300

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Abstract

Gram-positive *Staphylococcus aureus* is known to cause a variety of potentially fatal infections, especially in hospital environments. Through genetic changes and the use of specialised secretion systems, it has acquired resistance to a variety of antibiotics. The Type VII secretion system (T7SS) is one of the most interesting of these. Despite being crucial to the pathogenicity and survival of *S. aureus* during infection, T7SS is still only partially understood since some of its constituent proteins have unknown roles. T7SSs are frequently present in multidrug-resistant clinical strains of S. aureus, which are linked to serious infections with high fatality rates. The T7SSs are studied in the lab using the USA300 strain of S. aureus. In order to combat the *S. aureus* USA300 strain, we assessed the synergistic effects of 13 antimicrobial medications in combination with eight distinct plant extracts: Arjuna (*Terminalia arjuna*), Onion (*Allium cepa*), Bael (*Aegle marmelos*), Tulsi (*Ocimum sanctum*), Mango (*Mangi fera indica*), Amla (*Phyllanthus emblica*), Henna (*Lawsonia inermis*), and Lemon (*Citrus limon*). The disc diffusion method was used to test for antimicrobial susceptibility. Mueller-Hinton Agar (MHA) was used to create Petri plates, either with or without sub-inhibitory plant extract concentrations. Millimetres were used to measure the zones of inhibition. All studied plant extracts were found to be active against *S. aureus* USA300 in vitro. All extracts showed synergistic benefits with antibiotics, however the most notable synergism was shown with Arjuna, Tulsi, and Onion. Bael and Amla, on the other hand, showed little synergistic effects. Finding new synergistic combinations of plant-derived chemicals and antimicrobial medications was the main goal of this investigation. The results support further attempts to create natural antibacterial agents for alternative therapeutic approaches.

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Introduction

Traditional medicine relies heavily on medicinal plants, which have long been acknowledged as excellent sources of therapeutic substances that enhance human health and quality of life. Numerous plant species have been shown by science to have therapeutic qualities, mostly because of their bioactive chemicals that take part in biological processes necessary for the treatment and prevention of disease [1]. Medicinal plants are frequently the main, and perhaps the only, source of treatment for many populations, especially those in rural or indigenous contexts [2]. Their use is particularly important in underdeveloped nations where traditional medicines constitute a major part of primary healthcare and access to mainstream medicine may be restricted [3,4]. Infectious diseases continue to be a significant cause of morbidity and mortality, especially in areas with limited resources, despite advancements in pharmaceutical research and the creation of many antimicrobial medicines in recent decades [4]. The growing issue of antibiotic resistance exacerbates this difficulty. The genetic ability of microorganisms, particularly bacteria, to develop and spread medication resistance reduces the effectiveness of many traditional treatments [5]. Microbiology has done a lot of in vitro research on plants that are traditionally used in medicine, especially with regard to how they affect the growth of harmful bacteria. Nevertheless, little research has been done on the potential for synergy between traditional antibacterial medicines and plant extracts. In this study, we evaluated the in vitro synergistic interactions between certain antimicrobial medications and extracts of Arjuna (*Terminalia arjuna*) [6], Onion (*Allium cepa*) [7], Bael (*Aegle marmelos*) [8], Tulsi (*Ocimum sanctum*) [9], Mango (*Mangi fera indica*) [10], Amla (*Phyllanthus emblica*) [11], Henna (*Lawsonia inermis*) [12], and Lemon (*Citrus limon*) [13]. The Kirby-Bauer disc diffusion method was used to conduct the experiments against the *S. aureus* USA300 strain.

Materials and methods

Plant samples

Samples of *Terminalia arjuna, Aegle marmelos, Ocimum sanctum, Mangi fera indica,* and *Lawsonia inermis* were collected in June 2025 from Sant Shri Asaram ji Bapu Ashram, Botanical Garden, Ambala Road, Saharanpur-247001. Voucher specimens were prepared and deposited at the designated herbarium for future reference. The collected leaves were dried at 40°C and ground into fine powder using a mechanical mill [14,15]. In the same time, *Allium cepa, Phyllanthus emblica,* and *Citrus limon* were procured fresh from the local market and used in their natural form for extract preparation.

Preparation of plant extracts

Plant materials—dried (*Terminalia arjuna, Aegle marmelos, Ocimum sanctum, Mangi fera indica, Lawsonia inermis*) and fresh (*Allium cepa, Phyllanthus emblica, Citrus limon*) —were finely ground and extracted with 70% methanol. The initial extraction process lasted 48 hours, after which the mixtures were filtered [15]. The residual plant matter was re-extracted with fresh 70% methanol for an additional 24 hours, followed by a second filtration [15,16]. The combined methanolic extracts were then concentrated using a rotary evaporator at 45°C to remove the solvent. The crude extracts were stored in sterile bottles under refrigerated conditions until use. To determine the extract concentrations (mg/ml), the dry weight was calculated by complete evaporation of the remaining solvent [16].

Bacterial strains

Staphylococcus aureus USA300, a commonly used laboratory strain that was developed from ATCC1717, was utilized in this study. The ATCC1717 strain was obtained from the Department of Microbiology at NYU Grossman School of Medicine, located at 540 First Avenue, 2nd Floor, Lab 1-2, New York, NY 10016.

Media used

To maintain bacterial broth cultures, a nutrient broth was prepared using 0.5 g of NaCl, 0.5 g of peptone, and 0.3 g of beef extract per 100 ml of distilled water. For solid media, nutrient agar was formulated by adding 1.5 g of agar to the broth base [17].

Preparation of working slant

The *S. aureus* ATCC1717 stock culture was maintained at 4°C on semi-solid agar slants composed of 0.5% peptone, 0.3% beef extract, and 1.5% agar. For experimental use, a loopful of the culture was aseptically transferred from the stock to initiate active working cultures [17]. These slants were then incubated at 36 ± 1.0°C for 24 hours.

Broth preparation

Under sterile conditions, isolated colonies from the cultured *S. aureus* ATCC1717 slant were picked using a sterile inoculating loop and transferred into a cooled, autoclaved liquid broth medium containing 0.5% peptone and 0.3% beef extract [17,18]. The inoculated broth was then incubated at 36 ± 1.0°C for 24 hours, until visible turbidity indicated active bacterial growth.

Antimicrobial tests

Prior to evaluating the synergistic effects between plant extracts and antimicrobial agents, the Minimum Inhibitory Concentration (MIC) of each extract was determined against the *Staphylococcus aureus* USA300 strain. This was done by incorporating serial dilutions of the extracts into Mueller Hinton Agar (MHA) media NCCLS (2004a,b). Petri dishes containing various concentrations of the plant extracts (mg/ml), along with appropriate controls, were inoculated with approximately 10^4 CFU of *S. aureus* USA300 using a Steer's replicator and incubated at 37°C for 24 hours. The lowest concentration of each plant extract that visibly inhibited bacterial growth was recorded as the MIC, and the MIC_{90} (the concentration inhibiting 90% of isolates) was subsequently calculated. For synergism assays, one-fourth of the MIC_{90} was used as the sub-inhibitory concentration, as described previously [19]. These assays were performed on *S. aureus* USA300 and ATCC1717 strains using the disk diffusion method (Kirby-Bauer), following NCCLS (2004) guidelines on Mueller Hinton Agar (MHA). A total of thirteen antimicrobial agents were tested: Demeclocycline (DCC; 10 IU), Sarecycline (SCC; 1 µg), Paromoycin (PMC; 30 µg), Plazomicin (PZC; 10 µg), Tedizolid (TDZ; 30 µg), Bacitracin (BTC; 30 µg), Amoxicillin (AMN; 30 µg), Dicloxacillin (DAN; 10 µg), Nafcillin (NFN; 30 µg), Oxacillin (OCI; 30 µg), Indanyl Carbenicillin (ICI; 15 µg), Rifampin (RFM; 25 µg), and Trimethoprim (TMP; 5 µg). For each *S. aureus* USA300 strain, two sets of antibiograms were performed in duplicate: one on control plates containing plain Mueller Hinton Agar (MHA), and the other on MHA supplemented with one-fourth the MIC_{90} of the respective plant extract. After incubation at 37°C for 18 hours, the diameters (in mm) of the inhibition zones were measured and recorded [19,20].

Statistical analysis

Data obtained from the synergism assays were analysed using the Wilcoxon nonparametric test to compare the inhibition zone diameters (mm) generated by the disk diffusion method [20,21]. Statistical analysis was performed using Minitab Statistical Software, version 13.32 [22,23]. A p-value of less than 0.05 was considered statistically significant.

Results and discussion

Characteristics, MIC 90% (mg/ml) against *S. aureus* USA300 strain, and one-fourth the MIC 90% values obtained in the synergism assays for the plants and their respective extracts are presented in Table 1.

Anti-*S. aureus* activity was verified for all the plants. *T. arjuna* showed the highest activity, followed by *O. sanctum*; the lowest activity was recorded for *A. cepa*. The MIC 90% range was 0.38 mg/ml for arjuna and 17.5 mg/ml for *A. cepa* and it is not surprising the differences in the antimicrobial activity of plants tested, due to phytochemical properties and differences among species. Although the antimicrobial activities of *A. cepa*, *C. limon,* and *L. inermis*, have not been relatively high, synergism assays were carried out for them and the synergism rate of *A. cepa* was as high as that of *T. arjuna* Table 2.

Antimicrobial mechanisms of the drugs used here were variable and the protein synthesis inhibitors were those that presented strongest synergistic effect (5.0 extracts/drug) together with folic acid (4 extracts/drug) and bacterial cell wall synthesis (3.10 extracts/drug) inhibitors. Inhibitors of the nucleic acid synthesis (2 extracts/drug) showed weak synergism with plant extracts. Among the protein synthesis inhibitors, Demeclocycline showed synergism with all the extracts, followed by Sarecycline and Paromoycin. The synergistic capacity was promising for the extracts of some plants such as *T. arjuna*, *A. cepa*, and *O. sanctum*, which presented synergism with 11, 11, and 9 drugs, respectively; while *A. marmelos* and *P. emblica* showed synergism with only 4 and 3 drugs, respectively.

The synergism recorded here to plant extracts with weak action on *S. aureus* USA300 growth, such as *A. cepa*, is an important data since it showed a synergism profile similar to that of the *T. arjuna* extract, considered the most efficient *S. aureus* USA300 growth inhibitor in this study. Thus, the researchers should investigate the synergistic capacity of plant extracts or other natural products, independent of the antimicrobial activity they have. Therefore, the results of the present study seem to be promising and may enhance the natural products uses, showing the potential of these plants in the treatment of infectious diseases caused by *S. aureus.*

Table 1: Characteristics, Minimum Inhibitory Concentration (MIC_{90}) values, and sub-inhibitory concentrations (¼ MIC_{90}) used in synergism assays for the tested plants and their extracts.

Scientific name	Common name	Part of the plant used	Efficacy (%)	Extracts dry weight (mg/ml)	MIC 90% (mg/ml)	¼ MIC 90% (mg/ml)
Terminalia arjuna	Arjuna	Leaf	-	60.01	0.38	0.1
Allium cepa	Onion	Bulb	27.01	65	17.5	5.02
Aegle marmelos	Bael	Leaf	30.02	61.01	3.87	0.97
Ocimum sanctum	Tulsi	Leaf	49.01	131.01	0.53	0.14
Mangi fera indica	Mango	Leaf	21.85	12.01	2.26	0.57
Phyllanthus emblica	Amla	Fruit	-	95.12	5.07	1.26
Lawsonia inermis	Henna	Leaf	-	10.9	3.7	0.9
Citrus limon	Lemon	Fruit	28.02	12	3.59	0.89

(-): For non-dried (fresh) plant materials, the extract efficacy was considered as 100% of the original sample.

Table 2: Rate of synergistic activity between antimicrobial agents and plant extracts against Staphylococcus aureus USA300 strain using the Kirby-Bauer disk diffusion method.

Drug target	Drug	*Terminalia arjuna*	*Allium cepa*	*Aegle marmelos*	*Citrus limon*	*Mangi fera indica*	*Phyllanthus emblica*	*Lawsonia inermis*	*Ocimum sanctum*	*Synergism rate (extract/drug)*	*MEAN*
Protein synthesis	Demeclocycline	x	x	x	x	x	x	x	x	8	5.0
	Sarecycline	x	x	-	x	x	x	x	x	7	
	Paromoycin	x	-	x	x	x	x	x	x	7	
	Plazomicin	x	x	-	-	x	-	x	-	4	
	Tedizolid	x	-	x	x	-	-	x	-	4	
Cell wall synthesis	Bacitracin	x	x	-	x	x	-	-	x	5	3.10
	Amoxicillin	x	x	-	x	x	-	-	x	5	
	Dicloxacillin	x	x	-	-	-	-	x	-	3	
	Nafcillin	x	x	-	x	-	-	-	x	4	
	Oxacillin	x	x	-	-	-	-	-	-	2	
	Indanyl Carbenicillin	-	x	-	-	-	-	-	x	2	
Nucleic acids	Rifampin	-	x	-	-	-	-	-	x	2	2
Folic acid	Trimethoprim	x	x	x	-	-	-	-	x	4	4
Total	13	11	11	4	6	6	3	6	9		

X: synergism when p<= 0.05, (-) no synergism

Future studies on the chemical characteristics of extracts and active components should be carried out for each plant and antimicrobial property, since only crude extracts and their dry weight have been used in MIC determination (expressed in mg/ml) and synergism assays. In the current study, the plant extract antimicrobial activity against *S. aureus* USA300 strain was confirmed and synergism was possible with all the antimicrobial drugs tested. Demeclocycline showed synergism with all plant extracts; and the *A. cepa* extract, although with the lowest antimicrobial activity, presented a synergism profile similar to that of *T. arjuna*, whose extract showed a relatively high inhibitory capacity on *S. aureus* USA300 growth. The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and inhibitors of protein synthesis, however, the understanding of synergism mechanism is fundamental to development of pharmacological agents to treat diseases by *S. aureus* using medicinal plants.

Author declarations

Author contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Disclosures human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Conflicts of interest

No conflicts of interest.

Payment/services info

All authors have declared that no financial support was received from any organization for the submitted work.

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All authors have declared that they have no financial relationships at present with any organizations that might have an interest in the submitted work.

Other relationships

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