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Comparative In-*vitro* Antimicrobial Analysis between Plant Extract and Drugs used for *Staphylococcus aureus*

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Abstract

Searches for substances with antimicrobial activity are frequent, and medicinal plants have been considered interesting by some researchers since they are frequently used in popular medicine as remedies for many infectious diseases. The aim of this study was to verify the synergism between 13 antimicrobial drugs and 6 plant extracts–Guava (*Psidium guajava*), clove (*Syzygiumaromaticum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), and mint (*Mentha piperita*) – against *Staphylococcus aureus* strains, and for this purpose, the disk method was the antimicrobial susceptibility test performed. Petri dishes were prepared with or without dilution of plant extracts at sub-inhibitory concentrations in Mueller-Hinton Agar (MHA), and the inhibitory zones were recorded in millimeters. In vitro anti-Staphylococcus aureus activities of the extracts were confirmed through statistical analysis.

***Keywords*:** *Medicinal plants, drugs, Staphylococcus aureus, Disc diffusion*

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive, clustered, spherical-shaped bacterium; it is primarily a human and animal pathogen. It belongs to the family of Staphylococcaceae and the genus known as Staphylococcus. A Scottish surgeon, Sir Alexander Ogston (1881), while performing a procedure, discovered that Staphylococcus could cause wound infections in living organisms (Ogston, 1881). He introduced the term Staphylococcus for the genus in 1882, and Rosenbach (1884) detached the genus into *S. aureus* and *S. albus* (Ogston, 1882, Cowen *et al*, 1954). In 1939, Cowan distinguished *S. epidermidis* as a separate species based on coagulase testing (Cowan, 1939). Normally, *S. aureus* can also be found in healthy individuals (Taylor and Unakal, 2022). It does not cause any infection on healthy skin; however, upon entering the internal tissues or bloodstream, it may cause serious diseases (Turner *et al.*, 2019). *S. aureus* can cause minor skin infections such as impetigo, scalded skin, pimples, boils, abscesses, etc. It also causes life-threatening diseases such as meningitis, pneumonia, endocarditis, bacteremia, and sepsis (Diekema*et al.*, 2001, Appelbaum, 2006). The discovery of antibiotics helped to treat the infectious diseases caused by *S. aureus*. The antibiotic penicillin was discovered in 1928 by Sir Alexander Fleming. The purified form of penicillin came in 1941 and saved the lives of many war victims during World War II. Within 2 years of introducing penicillin, *S. aureus* resistance had emerged (Kirby, 1944). The first penicillin-resistant *S. aureus* strain was identified in 1942 (Rammelkamp and Maxon, 1942). The semisynthetic antibiotic methicillin was designed in 1950s, and methicillin-resistant *S. aureus* (MRSA) was clinically detected in 1960s (Jevons, 1961). The first MRSA strains were found in the United Kingdom, and this epidemic was primarily constrained to Europe. Soon after, MRSA was identified in the United States, Japan, and Australia.

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MRSA strains can produce a penicillin-binding protein (PBP) related with a diminished affinity for most semisynthetic penicillins. The PBP can be encoded by an obtained gene, mecA(Hartman and Tomasz, 1984, Reynolds and Brown, 1985). The methicillin-resistant genetic component (mecA) is carried on a mobile genetic element (MGE) selected staphylococcal cassette chromosome mec (SCCmec). The emergence of methicillin-resistant strains of staphylococci is because of the acquirement and inclusion of the mobile genetic elements into the chromosomes of the vulnerable strains.

Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups (Di Stasi 1996); and their use, especially in South America, contributes significantly to primary health care (Holetz*et al*. 2002). Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical industries have produced several new antimicrobial drugs in the last years, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento *et al*. 2000). In vitro studies on plants used in traditional medicine have been carried out in the field of microbiology, especially on pathogenic bacterial growth; and some of these studies were about the antimicrobial activity of *Psidium guajava L* (guava) (Holetz*et al*. 2002, Voravuthikunchai*et al*. 2004, Qadan*et al*. 2005), *Syzygiumaromaticum* (L)(clove) (Lopez *et al*. 2005), *Allium sativum L* (garlic) (Srinivasan *et al*. 2001, Benkeblia 2004), *Zingiber officinale Roscoe* (ginger), *Cymbopogon citratus* (lemongrass) (Cimanga*et al*. 2002) and *Mentha piperita L* (mint) (Tassou*et al*. 2000).

In the present study attempt has been made to compare the antimicrobial potential of these plant extracts with previously tested medicines employed against various bacteriogenic diseases.

Materials and Methods

Plant samples - *Psidium guajava,Syzygiumaromaticum, Allium sativum L,Zingiber officinale Roscoe, Cymbopogon citratus*and*Mentha piperita L*samples were collected in 2022 from an fieldsRaipur district, Chhattisgarh and the voucher specimens were identifiedat Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G). Their leaves were dried at 40oC and triturated in a mechanical mill. *A. sativum*and *Z. officinale* samples were obtained from the local commerce in the same year and were used in natura for the extracts' preparation.

Preparation of plant extracts - Plant material was dried, grounded, extracted with 70% methanol, and filtered after 48 h. The plant residue was re-extracted with addition of 70% methanol, and after 24 h it was filtered again. Combined filtrates were concentrated on a rotary evaporator at 45°C for methanol elimination, and the extracts were kept in sterile bottles under refrigerated conditions until use. The extracts' dry weight was obtained by the solvent evaporation and used to determine concentration in mg/ml.

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Bacterial strains -*S. aureus*(MTCC 7443) strains were obtained from MTCC, IMTECH, Chandigarh, India.The bacterial cultures were first inoculated in Nutrient Agar Medium (Doughari, 2006) and maintained in agar slants at 4ºC for further analysis.

Antimicrobial tests - Bacterial culture was incubated at 35±1^{0}C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6h). When a visible turbidity was obtained at the end of incubation time, the turbidity of bacterial suspension was adjusted against McFarland standard Tube [0.5] with sterile saline or broth. The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. The discs were prepared by impregnation as four discs. Then all the discs were dried at 40^{0}C and placed into the bacteria inoculated Petri dishes. Petri dishes, controls and with different concentrations of plant extracts (mg/ml), were inoculated with S. aureus strains (104 CFU) using a Steer's replicator and were incubated at 37°C/24 h. Thirteen drugs were evaluated: Penicillin (PEN; 10 IU), Oxacillin (OXA; 1 µg), Vancomycin (VAN; 30 µg), Ampicillin (AMP; 10 µg), Cephalothin (CFL; 30 µg), Cefoxitin (CFO; 30 µg), Chloramphenicol (CLO; 30 µg), Gentamicin (GEN; 10 µg), Netilmicin (NET; 30 µg), Tetracycline (TET; 30 µg), Erythromycin (ERI; 15 µg), Cotrimoxazole (SUT; 25 µg), and Ofloxacin (OFX; 5 µg).All Petri dishes after inoculation were allowed to dry for 15-20 min in room temperature. The plates were then incubated in an incubator set to 35±1^{0}C (Salie*et al*,, 1996). After 24 to 48h of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding callipers or a ruler, which was held on the back of the inverted Petri plate. All the experiments were performed in triplicates.

Statistical analysis- Results from antimicrobial analysis were subjected to statistical analysis using SPSS v16.2. Results were considered significant when $p < 0.05$.

Result

Characteristic antimicrobial activity of assays for the plants and their respective extracts are presented in Table I against *S. aureus* (MTCC 7443) strain. Anti-*S. aureus* activity was verified for all the plants. *S. aromaticum* showed the highest activity, followed by *P. guajava*; the lowest activity was recorded for*C.citratus*.The details of activity displayed by other medicinal plants are given in Table 1. Table 2 represents the antimicrobial activity of various drugs. Among the various drugs the highest activity observed was of Penicillin and lowest was recorded of Cotrimoxazole.

Discussion and Conclusion

The increasing occurrence, particularly in hospitals, of S. aureus resistant not only to methicillin but to a wide range of antimicrobial agents, including all kinds of β-lactams, has made therapy more difficult (Schito, 2006). Although strategies have been proposed as an attempt to control the spread (Blatnik and Lesnicar, 2006), the search for new ways to treat MRSA infections stimulates the investigation of natural compounds as an alternative treatment of these infections.In the present study, the analysis of the growth inhibition

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activity by the disk diffusion method showed that medicinal plants commonly used by traditional medical practitioners in were active againststrains of Staphylococcus aureus under test conditions with crude extract concentrations as high as 5 mg/ml. Among the plants tested highest activity was observed for *S. aromaticum*which is in equivalence with many of the antibiotic drugs tested and emerge as potential inhibitors of plant origin proving competitors for drug molecules.

Table 1: Antimicrobial activity of medicinal plants

Scientificname	Common Name	Parts Used	Zone of inhibition (in mm±SD)
Psidium guajava	Guava	Leaves	22.23±0.2
Syzygiumaromaticum	Clove	Flower buds	**26.05±0.4**
Allium sativum	Garlic	Flower buds	22.55±0.3
Zingiber officinale	Ginger	Rhizomes	23.30±0.4
Cymbopogon citratus	Lemongrass	Leaves	**20.32±0.3**
Mentha piperita	Mint	Leaves	24.45±0.5

Table 2: Antimicrobial activity of various drugs

Drug	Zone of inhibition (in mm±SD)
Penicillin	**29.03±0.4**
Oxacillin	26.04±0.5
Vancomycin	29.03±0.5
Ampicillin	22.04±0.6
Cephalothin	23.23±0.2
Cefoxitin	27.21±0.7
Chloramphenicol	23.34±0.4
Gentamicin	26.42±0.3
Netilmicin	22.04±0.3
Tetracycline	28.32±0.3
Erythromycin	23.22±0.4
Cotrimoxazole	**19.23±0.3**
Ofloxacin	24.35±0.2

References

- Ogston, A. (1881). Report upon micro-organisms in surgical diseases. *British medical journal*, *1*(1054), 369-b2.
- Ogston A. Micrococcus poisoning. *J. Anat. Physiol.* 1882; 16:526–567.
- Cowan, S. T., Shaw, C., & Williams, R. E. O. (1954). Type strain for Staphylococcus aureus Rosenbach. *Microbiology*, *10*(1), 174-176.
- Cowan, S. T. (1939). Classification of staphylococci by slide agglutination. *Journal of Pathology and Bacteriology*, *48*, 169-73.

- Taylor, T. A., &Unakal, C. G. (2022). Staphylococcus aureus. In *StatPearls [Internet]*. StatPearls Publishing.
- Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., ... & Fowler Jr, V. G. (2019). Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nature Reviews Microbiology*, *17*(4), 203-218.
- Diekema, D. J., Pfaller, M. A., Schmitz, F. J., Smayevsky, J., Bell, J., Jones, R. N., ... & SENTRY Participants Group. (2001). Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clinical Infectious Diseases*, *32*(Supplement_2), S114-S132.
- Appelbaum, P. C. (2006). MRSA—the tip of the iceberg. *Clinical microbiology and infection*, *12*, 3-10.
- Kirby, W. M. (1944). Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. *Science*, *99*(2579), 452-453.
- Rammelkamp, C. H., & Maxon, T. (1942). Resistance of Staphylococcus aureus to the Action of Penicillin. *Proceedings of the Society for Experimental Biology and Medicine*, *51*(3), 386-389.
- Jevons, M. P. (1961). 'Celebenin'-resistant staphylococci. *Bmj*, *1*, 124-125.
- Hartman, B. J., & Tomasz, A. (1984). Low-affinity penicillin-binding protein associated with beta-lactam resistance in Staphylococcus aureus. *Journal of bacteriology*, *158*(2), 513-516.
- Reynolds, P. E., & Brown, D. F. (1985). Penicillin-binding proteins of β-lactam-resistant strains of Staphylococcus aureus: Effect of growth conditions. *FEBS letters*, *192*(1), 28-32.
- Di Stasi, L. C. (1996). Plantasmedicinais: arte e ciência. *Um guia de estudointerdisciplinar-Editora da UNIVERSIDADE ESTADUAL PAULISTA-São Paulo-SP*.
- Holetz, F. B., Pessini, G. L., Sanches, N. R., Cortez, D. A. G., Nakamura, C. V., & Dias Filho, B. P. (2002). Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Memórias do Instituto Oswaldo Cruz*, *97*, 1027-1031.
- Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, *31*, 247-256.
- Voravuthikunchai, S., Lortheeranuwat, A., Jeeju, W., Sririrak, T., Phongpaichit, S., &Supawita, T. (2004). Effective medicinal plants against enterohaemorrhagic Escherichia coli O157: H7. *Journal of Ethnopharmacology*, *94*(1), 49-54.
- Qa'dan, F., Thewaini, A. J., Ali, D. A., Afifi, R., Elkhawad, A., &Matalka, K. Z. (2005). The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. *The American Journal of Chinese Medicine*, *33*(02), 197-204.
- Lopez, P., Sanchez, C., Batlle, R., &Nerin, C. (2005). Solid-and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *Journal of agricultural and food chemistry*, *53*(17), 6939-6946.
- Srinivasan, D., Nathan, S., Suresh, T., &Perumalsamy, P. L. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of ethnopharmacology*, *74*(3), 217-220.
- Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). *LWT-food science and technology*, *37*(2), 263-268.
- Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., ... &Vlietinck, A. J. (2002). Correlation between chemical composition and antibacterial activity of essential oils of

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Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 7, July 2022

some aromatic medicinal plants growing in the Democratic Republic of Congo. *Journal of ethnopharmacology*, *79*(2), 213-220.

- Tassou, C., Koutsoumanis, K., &Nychas, G. J. (2000). Inhibition of Salmonella enteritidis and Staphylococcus aureus in nutrient broth by mint essential oil. *Food research international*, *33*(3-4), 273-280.
- Avancini, C. A. M., Wiest, J. M., &Mundstock, E. (2000). Bacteriostatic and bactericidal activity of the Baccharis trimera (Less.) DC-Compositae decocto, as disinfectant or antisseptic. *ArquivoBrasileiro de MedicinaVeterinária e Zootecnia*, *52*, 230-234.
- Schito, G. C. (2006). The importance of the development of antibiotic resistance in Staphylococcus aureus. *Clinical microbiology and infection*, *12*, 3-8.
- Blatnik, J., &Lešničar, G. (2006). Propagation of methicillin-resistant Staphylococcus aureus due to the overloading of medical nurses in intensive care units. *Journal of Hospital Infection*, *63*(2), 162-166.