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Research Article

Isolation of Staphylococcus and gram-negative bacteria from the hospitalized area and screening bacteria against various plant extract

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INTRODUCTION

Staphylococcus aureus is a bacterium that is a member of the Firmicutes and is frequently found in the human respiratory tract and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine.

Staphylococcus was first identified in 1880 in Aberdeen, United Kingdom, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. This name was later appended to ***Staphylococcus aureus*** by Rosenbach who was credited by the official system of nomenclature at the time. It is estimated that 20% of the human population are long-term carriers of *S. aureus*˙ which can be found as part of the normal skin flora and in anterior nares of the nasal passages.*S. aureus* is the most common species of staphylococcus to cause *Staph* infections and is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies.*S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from the skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection.

Disease caused by Staphylococcus

Staphylococcus aureus is one of the leading causes of infections acquired in the community and after surgery or hospital. Around 30% of individuals carry S. aureus in their nose, pharynx or back of the throat and on their skin.

S. aureus causes numerous of infections at various sites of the body. Some of these include:

- **Skin infections** – S. aureus causes boils, furuncles, styles, impetigo and other superficial skin infections in humans.
- **Infections of surgical and trauma wounds** – Those with chronic illness, diabetes, traumatic injury, burns or immunosuppression are susceptible to the more severe skin, deeper tissue infections and deep abscesses.
- **Urinary tract infections**.
- Food poisoning and gastrointestinal tract infections may be caused by consuming food contamination.
- **Infected of organs-** include pneumonia (lung infection), osteomyelitis (bones infection), endocarditis (heart infections), phlebitis (infection of veins and blood vessels), mastitis (infection of breast and formation of abscesses) and meningitis (brain infections). These infections are more common in hospitalized patients rather than healthy individuals in the community.
- Infections from and on indwelling medical devices. These include infection of joint prostheses, cardiovascular devices and artificial heart valves. anemia and septicemia.
- Generalized life-threatening blood infections or Toxic shock syndrome (TSS), bacteria.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates came into existence soon after the introduction of methicillin. Historically, MRSA isolates have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes. However, in recent years, different strains with unique phenotypes have emerged in the community, and the reservoir of community-associated MRSA is rapidly expanding. Community-associated pathogens are likely to cause life-

threatening systemic infections, especially in children and elderly individuals, and may also cause serious skin and soft-tissue infections in healthy individuals. Compared with nosocomial strains, community-associated MRSA isolates are associated with increased virulence and currently are more likely to be susceptible to a variety of antibiotics. The epidemiological and microbiological differences between community-associated and nosocomial MRSA infections necessitate different strategies to prevent and treat the 2 types of infections. Vancomycin nonsusceptibility in *S. aureus* is on the increase, further complicating therapy.

- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen worldwide; MRSA infections are associated with increased morbidity and mortality, in comparison with other *S. aureus* infections. Over the past decade, the changing pattern of resistance in *S. aureus* has underscored the need for new antimicrobial agents. Once confined to healthcare-associated environments, MRSA has now migrated into the community. Community-associated strains share some characteristics with nosocomial strains but also differ in antimicrobial susceptibility and potential virulence. of concern is the probable increasing prevalence of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-intermediate *S. aureus* (VISA) MRSA strains in Europe, Asia, and the United States. Although 7 cases of infection with vancomycin-resistant *S. aureus* (VRSA) strains have been described in the United States, the clinical and epidemiological significance of this resistance phenotype is unclear at the present time
- Methicillin resistance in *S. aureus* involves an altered target site due to an acquired penicillin-binding protein (PBP 2a) with decreased affinity to β-lactams The *mecA* gene encodes this protein and is located on a mobile SCC*mec* cassette chromosome. This genetic element confers resistance to most currently available β-lactam antibiotics.

An antimicrobial agent-

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials (commonly known as antibiotics) are used against bacteria and antifungals are used against fungi. They can also be classed according to their function. Antimicrobials that kill microbes are called microbiocidal; those that merely inhibit their growth are called microbiostatic. Disinfectants such as bleach are non-selective antimicrobials.

Multi-Trait of Staphylococcus-

A. Staphylococcus aureus is an opportunistic pathogen of a human being.
B. A common sore throat is caused by Staphylococcus aureus.
C. Drug resistance is very common in clinical isolation of Staphylococcus aureus.
D. Therefore there is a demand for new drugs/molecules which has inhibitory activity towards S. aureus and have no side effects.

MATERIAL AND METHODS-

- Isolation of Staphylococcus and gram-negative bacteria from the patient serum.
- Identified that S. aureus and gram-negative bacteria.
- Antibiotics Resistance toward commonly used antibiotics against *Staphylococcus bacteria.*
- Prepare the antibacterial activity of plant extract-

1. Thuja (Orientalis).
2. Guava (Psidium Guajava).
3. Castor (Ricinus communis).

Table 1 Selection of best plants extract showing highest activity

S. No.	Used of media	Chemical	Plant Extract	Antibiotics
1	Nutrient agar	Diethyl ether	Thuja (Orientalis) leaf	Penicillin-G
2	Mannitol salt agar	Ethanol	Guava (psidium guajava)leaf	Amoxicillin
3	Muller Hinton Agar	Methanol	castor (Ricinus communis)leaf	Ciprofloxacin
		hexane,		Norfloxacin
		diethyl ether		

Isolation of Staphylococcus aureus-

Specimens were collected using a rayon-tipped swab with Amies charcoal transport medium. The anterior nares were sampled by rotating the swab tip in both nostrils, and the throat was sampled by rotating the swab tip on both tonsils. All swab samples were incubated for 24 hours at 37°C in a shaker (100 rpm) under aerobic conditions in 3 ml enrichment broth Isolated Staphylococcus aureus with the following composition: 15.0 g protease peptone, 5.0g yeast extract (Oxoid), 25.0g NaCl, 10.0g mannitol, (final pH, 7.0 ± 0.1). A portion (10 µl) of the broth was then plated on a mannitol salt agar plate were incubated at 37°C for 24-hours. After the incubation of colonies was appears on mannitol salt agar plate.

Identification of Gram staining-

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by the Safranin. There are four basic steps of the Gram stain:

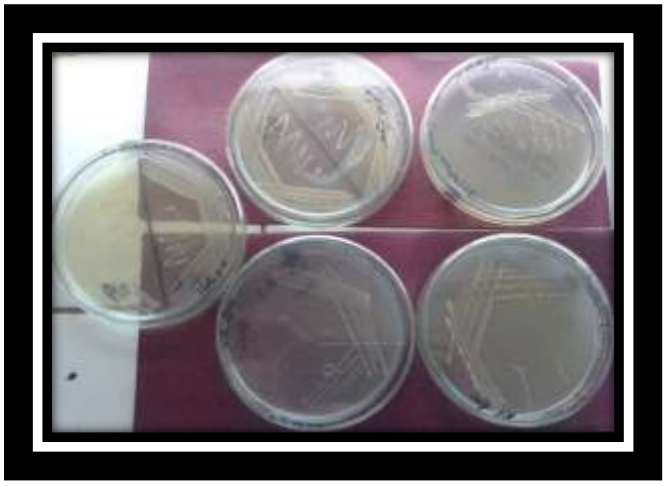
- Take the 24 Hours old fresh culture and contain the smear applying an one drop of primary staining (crystal violet) after heat-fixed smear of a bacterial culture. Heat fixing

kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the staining procedure.

- The addition of iodine, which binds to crystal violet and traps it in the cell,
- Rapid decolorization with alcohol or acetone.
- Counterstaining with safranin. Carbolfuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.

Purification and maintenance-

This was done by repeated subculturing on nutrient agar plates. The colonies from MSA plates were streaked on nutrient agar plates and incubated at 37°C for 24 Hours in order to obtain isolated colonies of pure culture. Subculturing of purified colonies was also done on nutrient agar plate every seven days.



Maintenance of Staphylococcus aureus

Antibiotic Resistance-

It was done as per according to ***National Council For Clinical and Laboratory Standard*** (NCCLS) Protocols by the disc diffusion method. The testing culture suspension was prepared in 5 ml normal saline (0.89%NaCl 0.2 N) and 100µl was spread on Muller Hinton Agar plate with a sterile glass spreader. A disc of antibiotic was kept carefully on the center of medium pour plate the lown using a flamed forceps. The plate was incubated at 37°C for 24 hours and after zone of inhibition was measured in mm by the scaled rod. This was compared with the standard values given in the NCCLS chart. If the zone was found to be greater than the mentioned values then the test culture was said to be sensitive otherwise resistant on intermediate.

Preparation of plant extracts-

Three plants are used for the preparation of plant extracts such as thuja (orientalis), guava (psidium guajava) and castor (Ricinus communis) were selected. Leaf extracts were prepared in one polar and one nonpolar solvent. The polar solvent used was ethanol and the nonpolar solvent was being used either.

Procedure- 5g leaf tissue was crushed in a sterile mortar with a sterile pestle using 10ml of solvent at a time. The solution was filtrated through the whatsmans filter paper collected in a freshly sterilized volumetry flask or graduated cylinder and final volume of extract was makeup to 5ml.

Assay for antimicrobial potential of plant leaf extracts-

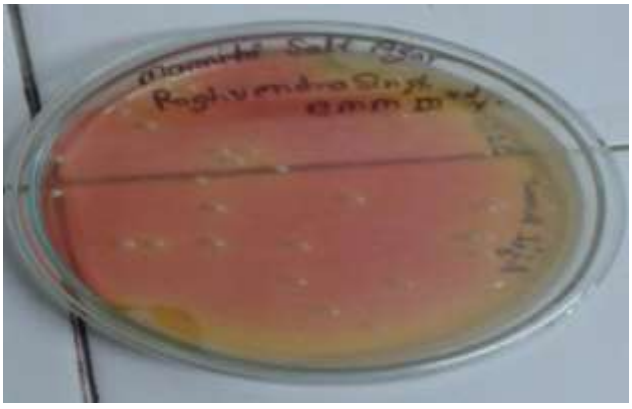
The cut the 5mm whatman's filter paper disc (presterilized) was dipped in the plant extract to be tested allowed to dry for 5 minutes under the laminar air flow kept on the central lid of Petri plate. Then this was kept on the lawn of Staphylococcus aureus prepared on Muller Hinton agar and plate were incubate at 37°C for 24 hours 'The zone of inhibition was measured in the same way as with the antibiotics mentioned in the section above.

RESULT-

Isolation of S. aureus bacteria- Five S. aureus culture isolates from two places of Kanpur, Uttar Pradesh, India(1) SCMAT College Kanpur (2) GSVM Kanpur. Those Cultures are isolated to Manital Salt Agar Medium and confirm them bacteria to gram staining and culture characteristics.

Table 2 Isolation of culture and identified the gram staining

S. No.	Culture No.	Isolated place	Culture Characteristics	Gram Staining	
1	Staph-1	SCMAT College, Kanpur	Dark Yellow colonies, small colonies	Violet colour	Circular cluster
2	Staph-2	SCMAT College, Kanpur	Dark yellow large colonies, nonmucoid	Violet colour	Small coccus cluster
3	Staph-3	SCMAT College, Kanpur	Light yellows	Violet colour	Coccus margins
4	Staph-4	GSVM, Kanpur	Red colonies	Violet colour	Rod coccus, cluster
5	Staph-5	GSVM, Kanpur	Dark yellow, mucoid,	Violet colour	Coccus grapes like



Antimicrobial activity of Guava-

To determine the antimicrobial potential activity of guava

(Psidium guajava) leaf extracts against two gram-negative bacteria (Escherichia coli and Salmonella enteritidis isolated from hospitalized patients) gram-positive bacteria (Staphylococcus aureus) which are causes some of the foodborne disease, UTI, GIT infection, wounds, boils and spoilage of vegetables. The guava leaves were extracted in four different solvents of increasing polarities (hexane, diethyl ether, ethanol, and water). The efficacy of these extracts was tested against those bacteria through a well-disc diffusion method employing 50 µL leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 11.0 mm against S. aureus, respectively. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. Antimicrobial activity of Castor (Ricinus communis)

Ricinus communis L. (*R. communist*) belongs to family Euphorbiaceae, is a soft wooden small tree, widely spread all through the tropics and temperate regions of the world. The *R. communis* methanolic leaf extract showed maximum (20.7 mm) antibacterial activity as compared to ethanolic and aqueous extracts against *S. aureus*. The ethanolic leaf extract showed a maximum zone of inhibition (18 mm) against *S. aureus* The water extract showed the lowest antibacterial activity against all the Gram positive and Gram negative bacterial strains as compared to the methanol and ethanol leaf extracts. *R. communis* is effective even at low concentration against bacterial and fungal pathogens. This work provides a scientific validation to *R. communis* in having the potential to be a good drug.

Zone of inhibition of Guava and thuja leaf extract

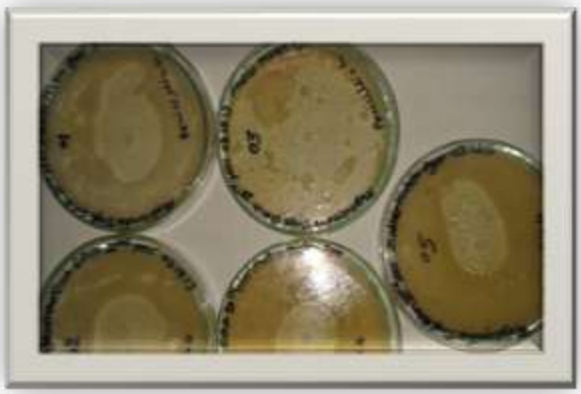
Antimicrobial activity of Thuja extract

Plant-derived bioactive substances are a good source of medicines that play a significant role for human health and also used against different types of microbial diseases. Thuja orientalis (commonly- morpankhi, family- Cupressaceae) is an evergreen and monoecious tree or shrub has been used in the different activity that is, antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic (Yeung, 1985). The phytoconstituents of T. orientalis such as flavonoids and terpenoids showed the biological activities (Hassanzadeh et al., 2001). Various phytochemical compounds had been isolated from the different parts of platycladus orientalis such as flavonoids from leaves, mono and sesquiterpenoids in essential oils of different parts of the plant (Yan-hua et al., 2006), some labdane and isopimarane diterpenoids from pericarpes and leaves (Kuo et al., 1990; Koo et al., 2002), two monolignol derivatives from pollens (Ohmoto et al., 1988). The most beneficial chemical compounds of T. orientalis oil are carnphor, fenchone, isothujone and thujone (Asili et al., 2007) Thuja leaf.

Table 3 Sensitivity/Resistance of S. aureus against to some antibiotics by disc diffusion method.

S. No.	Use of Antibiotics	Culture No.	Zone of Inhibition (mm)	Sensitivity/ Resistance
1	Penicillin-G 10mg/ml	Staph-1	35	S
		Staph-2	34	S
		Staph-3	35	S
		Staph-4	30	S
		Staph-5	25	S
2	Amoxicillin -10mg/ml	Staph-1	30	S
		Staph-2	17	**R**
		Staph-3	20	S
		Staph-4	35	S
		Staph-5	23	S
3	Ciprofiloxacin 10mg/ml	Staph-1	46	S
		Staph-2	45	S
		Staph-3	42	S
		Staph-4	43	S
		Staph-5	38	S
4	Norfloxacin 10mg/ml	Staph-1	36	S
		Staph-2	38	S
		Staph-3	35	S
		Staph-4	30	S
		Staph-5	28	S

A



B

Zone appears on S.aureus culture plates with use of Amoxicillin (A) and Penicillin-G (B) antibiotics.

Table 4 Inhibitory activity of different plant extract against S. aureus by disk diffusion method.

S.No.	Culture No.	Use of Plant Extract	Solvent use for Extraction	Zone of Inhibition (mm)
1	Staph-1	Thuja	Ethanol	09
			Diethyl ether	14
		Castor	Ethanol	00
			Diethyl ether	00
		Guava	Ethanol	07
			Diethyl ether	07
2	Staph-2	Thuja	Ethanol	07
			Diethyl ether	00
		Castor	Ethanol	00
			Diethyl ether	12
		Guava	Ethanol	13
			Diethyl ether	15
3	Staph-3	Thuja	Ethanol	08
			Diethyl ether	00
		Castor	Ethanol	07
			Diethyl ether	00
		Guava	Ethanol	00
			Diethyl ether	07
4	Staph-4	Thuja	Ethanol	12
			Diethyl ether	00
		Castor	Ethanol	08
			Diethyl ether	08
		Guava	Ethanol	07
			Diethyl ether	07

CONCLUSION-

Those results are so the best inhibition of S. aureus to use the Plant-derived bioactive substances are a good source of medicines that play a significant role for human health and also used against different types of microbial diseases. Thuja orientalis (commonly-morpankhi, family- Cupressaceae) is an evergreen and monoecious tree or shrub has been used in the different activity that is, antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic. All those selected the Plant Extract Inhibited the disease in a human being to be the use of a chemical solvent. All those chemical compound if used the small value of solvent and mix the plant extract so do not cause the Staphylococcus aureus.

DISCUSSION-

The S. aureus is an ubiquitous bacteria that is leading the superficial, UTI, Throat infection in human at the clinical environment for decades and also most important caused of food-borne disease in human. This result shows the widely used of antiaureuses and decrease the Nosocomial Infection (Hospital-acquired infection) for Human, This plant extract used the small value of natural-chemically products for packs fruit and other drinkable product this product more effective for human.

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