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PSEUDOMONAS AERUGINOSA PIGMENT- BIO COLOURS AS MOST POTENT ANTIBACTERIAL AGENT AGAINST S.AUREUS ISOLATE FROM CURRENCY NOTES

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ABSTRACT
Pseudomonas aeruginosa common pathogenic bacteria belonging to family *pseudomonadaceae* is a gram negative, rod shaped bacterium that causes diseases in humans as well as animals and mostly pathogenic to plants. *P.aeruginosa* is found in terrestrial and aquatic environment especially in moist environment of hospitals resulting in Hospital Acquired Infections. The main aim of the current study was to determine the antibacterial activity of crude acetone pigment extract of Pseudomonas *aeruginosa* against Staphylococcus aurous isolated from currency notes. The currency note was collected from the petty shop of a crowded area in a sterile zip lock cover. It was then transferred to Nutrient broth for the processing of the sample. The sample was observed under microscopy and processed by inoculating into the nutrient broth and incubated for 24 hours at 37°C and broth culture was centrifuged followed by inoculation in to various types of media. The isolate was identified as *Staphylococcus aureus* by morphological, cultural, biochemical characters. The bacterial pigment was extracted by sub culturing Psuedomonas *aeruginosa*. *Pseudomonas aeruginosa* was inoculated in to conical flask containing nutrient broth and incubated for 24hrs to 72 hrs in rotory shaker for production of pigment. The UV spectroscopic study was done to estimate the peak value of pigmented broth. The crude acetone pigment extract was prepared and assessed for antibacterial activity. The crude acetone pseudomonas pigment extract exhibited activity against *s.aureus* isolated from currency note which was found to be greater at 500µl with value of 19mm and minimum at 62.5 µl with value of 15mm.

KEYWORDS: Cetrimide agar, spectrophotometer, currency note.

INTRODUCTION
*Pseudomonas aeruginosa***,** common pathogenic bacteria belonging to family *pseudomonadaceae* is a gram negative, rod shaped bacterium that causes diseases in humans as well as animals and mostly pathogenic to plants. *P.aeruginosa*is found widely in terrestrial and aquatic environment especially in moist environment of hospitals resulting in Hospital Acquired Infections. This is a facultative anaerobe as it tends to get adapted to partial or total oxygen depletion. An important advantage of *Pseudomonas aeruginosa* is its ability to produce and release compounds with inhibitory activity on other bacteria, fungi, protozoa etc. The special characteristic feature of pseudomonas is the production of water - soluble, blue green pyocyanin pigment which is a secondary metabolite. This pyocyanin pigment produced by pseudomonas is a vital and potential factor that would enhance the survival *of P.aeruginosain* growing dense colonies by increasing its capacity to compete with other microbes or sometimes by lysing them that is it has got an antagonistic activity against bacterial and fungal species. *Pseudomonas* also possesses degradative properties and plays a vital role in environmental cleanup as well as possesses the opportunistic pathogenic abilities and hence termed as opportunistic bacteria.

It is a slender, gram- negative bacillus, 1.5-3 × 0.5 µm in size. Pseudomonas species are actively motile by polar flagellum. They are non- capsulated but many strains have a Mucoid slime layer. Mucoid strains, particularly isolates from cystic fibrosis patients, have an abundance of extracellular polysaccharides composed of alginate polymers. This forms a loose capsule, i.e; glycocalyx in which micro colonies of the bacillus are enmeshed and protected from host defences. The pseudomonas species are not active fermenters of carbohydrates (CHO) and produces only acids not gas in the glucose. This is lactose negative. The pseudomonas species are the only Gram negative bacilli having both catalase and oxidase as positive.

This is an obligate anaerobe which has a wide range of temperatures ranging from 6 - 42°C whereas the optimal temperature remains 37°C. This exhibits a high degree of

resistance to chemical agents. The ability to grow even at 42°C along with the pyocyanin production is enough to distinguish the pseudomonas aeruginosa form other pseudomonas species.

Grows well producing large and opaque colonies, mostly irregular colonies, with a distinctive, musty mawkish or earthy smell on ordinary media and iridescent patches with a metallic sheen are seen in cultures with crystals beneath the patches on Nutrient agar. It forms non-lactose fermenting colonies on the MacConkey medium. In Blood Agar Many strains are hemolytic while the pseudomonas forms a dense turbidity with surface pellicle into the broth. Mineral salt medium and peptone water favors better support for the growth of pigment whereas Mannitol which includes broth and broth malt with cooked meat extract shows less production of pigment for the incubation period and temperature of the same.

One of the vital things of the current era is *money* which facilitates the need and this includes both the currency notes and the coins. The exchange of currency notes occurs with the exchange of goods and so does the microbial population on them. As the notes and coins gets exchanged from one individual to the other the microbial contamination increases. This is one of the routes of microbial contaminants to humans. These exchanged notes might contain the pathogenic bacteria which may be the reason for the rate of increase in various infectious diseases. The transmission may occur through sneezing, coughing, touching, or by the vendors handling the currency notes hence, these contaminated notes may serve as the source for entero pathogens causing food-poisoning.

PIGMENT PRODUCTION

The Pseudomonas *genus* produces different number of extra-cellular pigments of which phenazines comprise the most prominent one. The important notable feature of *Pseudomonas aeruginosa* is the production of soluble blue green phezanine compound pyocyanin pigment and the yellow green fluorescent pigment which is pyoverdine. The pyocyanin had been used as a reversible dye with a redox potential similar to that of menaquinone from the beginning.Thepyocyanin pigment phenazine-based has a particular interest for its capability to generate reactive oxygen species (ROS). These phezanineRedox potential pigments are involved in virulence and iron acquisition. Pyocyanin also has antibiotic activity towards different microorganisms. This phezanine compound, pyocyanin possess an inhibitory action as a result of its unique redox potential. One general characteristic redox activity leads to the antagonistic effect of almost all of the phezanine derivatives.

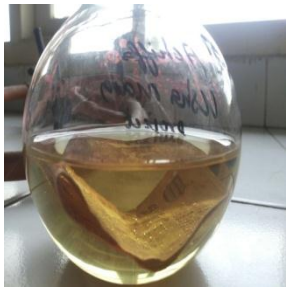
METHODOLOGY

Sample collection

The currency note was collected in crowded area from petty shop in zip lock cover using sterile hand gloves and transferred to sterile broth for sample processing.

Sample processing

The sample was then processed by inoculating into the nutrient broth and incubated for 24 hours at 37°C for determining the growth of organism followed by centrifugation. The organism in the sample was then identified by microscopic, cultural and biochemical tests as Staphylococcus aureus.



Currency note in nutrient broth

Identification of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa was identified by Gram staining, Hanging drop, catalase, and oxidase tests. The cultural characteristics and biochemical characters were performed to identify *Pseudomonas aeruginosa*.

Inoculation of Pseudomonas aeruginosa in to Nutrient broth.

Incubation of pigmented broth in rotary shaker at 37◦c.

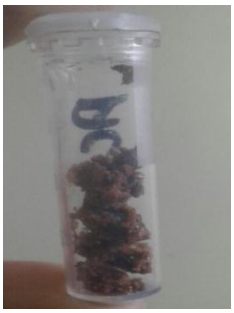
Pigment production after 24 hours.

Pigment production after 48 hours.

Filtration of centrifuged supernatant broth.

Extraction of pigment

The pigmented broth was transferred to sterile tubes and centrifuged for 30 mins at 1500 rpm. The supernatant was filtered by using sterile whats Mann filter paper. The filtrate was extracted using acetone solvent. The filtrate was evaporated to extract crude pigment and stored in vials for antibacterial activity.



Crude acetone pigment extract.

Antibiotic sensitivity test for *Staphylococcus aureus* isolate

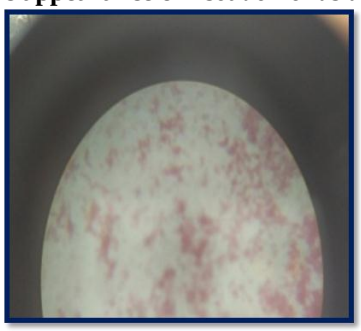
Staphylococcus aureus isolate was sub cultured and lawn was prepared. The antibiotic discs were placed and incubated at 37◦c for 24 hrs. The plates were observed for zone formation.

Antibacterial Activity of crude pigment extract

The antibacterial activity of crude acetone pigment of Pseudomonas aeruginosa was determined by inoculating *Staphylococcus aureus* isolate in to Nutrient broth and incubated for 24 hrs at 37◦c.The turbidity of broth was compared to 0.5 N McFarland solutions. The lawn was prepared using *Staphylococcus aureus* isolate on Muller Hinton agar. The wells were cut using sterile well puncher and one milli gram of pigment extract was suspended in 100 µl of acetone and 900 µl nutrient broth. Different concentrations of pigment extracts ranging from500, 250, 125, 62.5 µl were loaded in to wells using water as control. Muller Hinton agar plate was incubated at 37 ◦c for 24 hrs and observed for Zone formation.

RESULTS

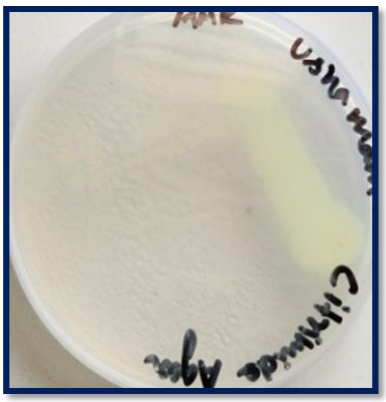
Microscopic appearance of *Pseudomonas aeruginosa*



Gram negative bacilli – *P.aeruginosa.*

COLONY MORPHOLOGY

Green pigmented colonies of *P. aeruginosa* on Nutrient agar.



Colonies of *P.aeruginosa* on Cetrimide agar.

Preliminary tests for *Pseudomonas aeruginosa*

Sl.no	Tests	Results
1.	Gram staining	Gram-Positive bacilli
2.	Motility	Motile
3.	Catalase	Postive
4.	Oxidase	Positive

Catalase positive – Pseudomonas aeruginosa.

Oxidase positive – *P.aeruginosa.*

Colony characteristics of *Pseudomonas aeruginosa*

Sl.no	Colony morphology	Results
1.	Size	1-2 mm
2.	Margin	Entire
3.	Shape	Circular
4.	Opacity	Opaque
5.	Consistency	Smooth
6.	Elevation	Convex

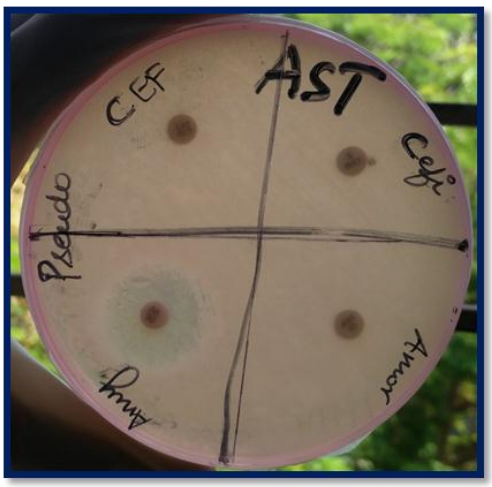
Biochemical charactersof *Pseudomonas aeruginosa*

Sl. No	Tests	Results
1.	Indole	Negative
2.	Methyl red	Negative
3.	Voges – Proskauer	Negative
4.	Citrate	Positive
5.	Urease	Positive
6.	Triple Sugar Iron	K/K
7.	Nitrate	Positive

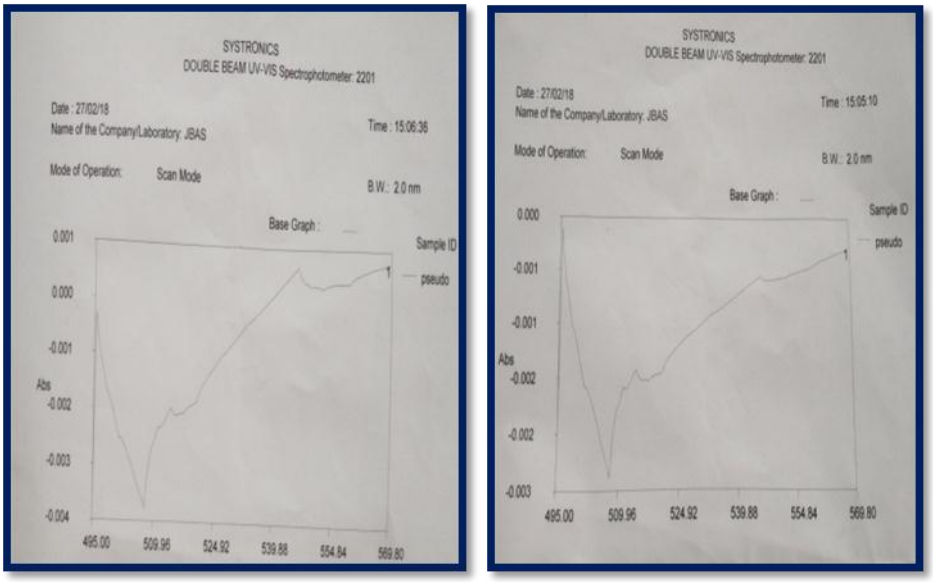
Oxidative Fermentation test for*P.aeruginosa.*

Antibiotic Sensitivity Test – *Pseudomonas aeruginosa*

Pseudomonas aeruginosa was found to be highly sensitive against the antibiotic Amikiacin with zone formation of 25 mm diameter. It was resistant to Amorphicilin, Ceftriaxone, Cefixime.



Sl.no	Antibiotics	Zone of Inhibition
1.	Amorphicilin	Nil
2.	Amikiacin	25 mm
3.	Ceftriaxone	Nil
4.	Cefixime	Nil



UV visible spectrophotometric analysis of pigmented broth of *P.aeruginosa.*

Well Diffusion Method for *P.aeruginosa* – Antibacterial Activity.

DISCUSSION

*Pseudomonas aeruginosa***,** common pathogenic bacteria belonging to family *pseudomonadaceae* is a gram negative, rod shaped bacterium that causes diseases in humans as well as animals and mostly pathogenic to plants. It is a slender Gram negative bacilli, motile, catalase positive, oxidase positive, produces green pigment on Nutrient agar and nonlactose fermenters on MacConkey agar. The main aim of this study was to extract pigment and determine the antibacterial activity against *Staphylococcus aureus* isolated from currency notes exchanged among the crowded population. This study showed that crude acetone pigment extracts exhibited most potent antibacterial activity against *Staphylococcus aureus*isolate by performing well diffusion using different concentrations of crude pigment extract ranging from 500µl to 62.5 µl exhibiting inhibitory values of 22mm, 19 mm, 17 mm and 15 mm respectively.

The isolate from currency note was examined microscopically by Gram staining. Gram staining revealed that the note sample was found to contain Gram positive cocci in clusters. The sample was inoculated in to Nutrient agar and Mannitol salt agar. Golden yellow colonies and yellow colonies were observed on nutrient agar and Mannitol agar. Coagulase and DNAse test was performed and found tu be positive. The antibiotic sensitivity test was performed for Staphylococcus aureus isolate and was found to be highly resistant to Pencillin. The isolate was found to be sensitive to Erythromycin followed by clindamycin and vancomycin with zone formation with values of 25mm, 17mm and 11 mm. The crude acetone pigment was used against *staphylococcus aureus* in different concentrations ranging from 500µl to 62.5 µl by well diffusion method. Maximum inhibition was found to be at different concentrations were found to be 22mm, 19 mm, 17 mm and 15 mm respectively.

DISCUSSION

*Pseudomonas aeruginosa***,** common pathogenic bacteria belonging to family *pseudomonadaceae* is a gram negative, rod shaped bacterium that causes diseases in humans as well as animals and mostly pathogenic to plants. It is a slender Gram negative bacilli, motile, catalase positive, oxidase positive, produces green pigment on Nutrient agar and nonlactose fermenters on MacConkey agar. The main aim of this study was to extract pigment and determine the antibacterial activity against *Staphylococcus aureus* isolated from currency notes exchanged among the crowded population. This study showed that crude acetone pigment extracts exhibited most potent antibacterial activity against Staph*ylococcus aureus* isolate by performing well diffusion using different concentrations of crude pigment extract ranging from 500µl to 62.5 µl exhibiting

inhibitory values of 22mm, 19 mm, 17 mm and 15 mm respectively.

SUMMARY AND CONCLUSION

*Pseudomonas aeruginosa*is a Gram negative slender bacilli, aerobic. Catalase and oxidase positive. It produces two kinds of pigments such as pyocyanin and pyoverdine. The pigments exhibited antimicrobial properties. *Pseudomonas aeruginosa* sub culturedand inoculated in to Nutrient broth followed by incubation at 37◦c for 48 hrs in rotary shaker for production of pigment. The pigmented broth was centrifuged at 1500 rpm for 30 mins. The supernatant was filtered using sterile whatmann filter paper.. The filtrate was mixed with acetone and kept in oven overnight to obtain crude extract. The crude extract was stored in sterile storage vials for antibacterial study. The currency note was collected from petty shop in crowded area and transferred to sterile Zip lock cover using sterile hand gloves. The currency note was transferred to nutrient broth in flask and incubated at 37◦c. The turbidity was observed and microscopic examination was done by Gram staining technique and found to be Gram positive cocci in clusters. The broth culture was inoculated in to Nutrient agar and Mannitol salt agar and incubated for 24 hrs at 37◦c. Coagulase and DNAse tests were performed to differentiate *Staphylococcus spp*. Antibiotic sensitivity tests was done to find out sensitivity of *Staphylococcus. aureus* isolate to antibiotics. The isolate was found to be highly resistant to Pencillin and sensitive to Erythromycin. The crude acetone pigment was used against *staphylococcus aureus* in different concentrations ranging from 500µl to 62.5 µl by well diffusion method. Maximum inhibition was found to be at different concentrations were found to be 22mm, 19 mm, 17 mm and 15 mm respectively. The current study reported that currency notes plays a vital role in transmission of infections from person to person as currency note is exchanged among the people in society. It was concluded that Currency note carries microbes which causes infections. The pigment extract acts as a novel bio colour against Staphylococcus. aureus isolated from currency note.

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