EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
528-532
Year: 2018

MICROCOCCUS ROSEUS PIGMENT- BIOCOLOUR AS NOVEL ANTIBACTERIAL AGENT AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATE FROM CURRENCY NOTES

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Article Received on 06/03/2018 **Article Revised on 27/03/2018** **Article Accepted on 17/04/2018**

ABSTRACT
Micrococcus is a genus of bacteria in the *Micrococcaceae* family. Micrococcus roseus widely range of environments including water, dust and soil. Micrococci species are Gram positive cocci and size ranges from about 0.5-3 micrometres in diameter and appear in tetrads. Natural pigments are extracted not only from fruits, vegetables and roots. The synthetic colours are highly toxic and expensive. It is essential to produce Biocolors. Pigments are bio active secondary metabolite in microbes because of Carotenoids effect on UV radiation of sun and reactive oxygen species. Microorganisms are found in paper notes because of the rough surface which allows them to settles for long periods. The level of contamination depends on how long the note should been in circulation with one hand to another, The microorganisms has capacity to absorb moisture and its texture. Microorganisms present for a long time for production of molecules like antibiotics, enzymes, It is more stable and soluble. 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 19 mm and 15 mm at 500 µl and 250 µl . The isolate was found to be resistant to pigment extracts at concentration range of 125 µl and 62.5 µl.

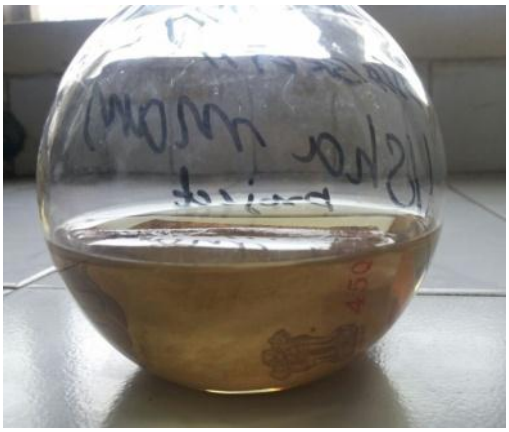
KEYWORDS: Muller Hinton agar, Carotenoids Pigment, Spectrophotometer.

INTRODUCTION
Micrococcus is a genus of bacteria in the *Micrococcaceae* family. *Micrococcus* widely range of environments including water, dust and soil. *Micrococci* species are Gram positive cocci and size ranges from about 0.5-3 micrometres in diameter and appear in tetrads. Species of *Micrococcus* such as *M. Luteus* yellow colour pigment when grown on mannitol salt agar .Isolates of *M. Luteus* have beenfound to overproduce riboflavin. Natural pigments are extracted not only from fruits, vegetables and roots. The synthetic color sare highly toxic and expensive. It is essential to produce Biocolors.Pigments are bio active secondary metabolite in microbe .because of Carotenoids effect on UV radiation of sun and reactive oxygen species. They act as sun protector factors by light absorption at 350-500 nm.Microorganisms are found in paper notes because of the rough surface which allows them to settles for long periods .The level of contamination depends on how long the note should been in circulation with one hand to another, The microorganisms has capacity to absorb moisture and its texture .Microorganisms present for a long time for production of molecules like antibiotics, enzymes,. It is more stable and soluble. They grow rapidly and then lead to high productivity. *Micrococcus roseus* is a Gram positive bacterial cell that grows in the tetrad arrangements. This is the normal habitat of skin, soil, andwater.It produces the Carotenoids pigment .It is an aerobic bacteria. Microorganisms are ubiquitous and assumed to be found on currency notes and coins are exchanging in society by people from various places such as bus etc.These are possibilities of spreading communicable and non-communicable diseases from infected persons ,carries and health care workers. The microbial load can be evaluated so as to determine the degree of pathogenecity to provide public awareness on handling the currency notes and coins by the currency handlers. *Micrococcus roseus* is Gram positive cocci in tetrads. It is catalase and oxidase positive, aerobic and facultative anaerobes.

MATERIALS AND METHODS
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 *Micrococcus roseus*

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

Inoculation of *Micrococcus roseus* into nutrient broth.

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

Pigment production after 48 hours of inoculation.

Crude pigment extract.

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.

Antibiotic sensitivity test for *Staphylococcus aureus* isolate

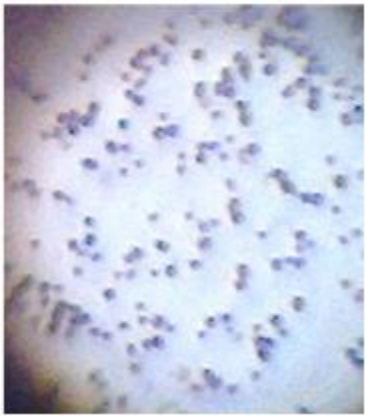
Staphylococcus aureus isolate was sub cultured and lawn was prepared. The antibiotic discs were placed and incubated at 37◦c for 24 hrs. The plates were observed for zone formation.

Antibacterial Activity of crude pigment extract

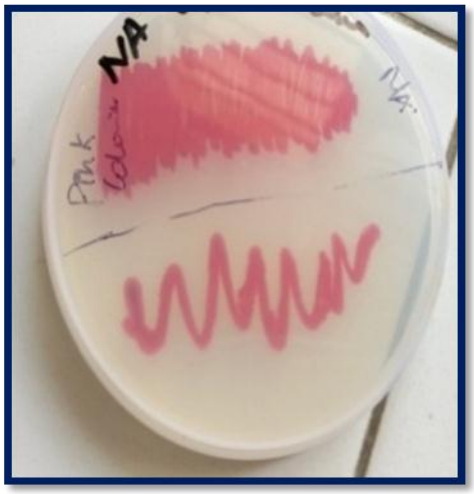
The antibacterial activity of crude acetone pigment of ***Micrococcus roseus*** was determined by inoculating *Staphylococcus aureus* isolate in to Nutrient broth and incubated for 24 hrs at 37◦c .The turbidity of broth was compared to 0.5 N McFarland solutions. The lawn was prepared using *Staphylococcus 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 *M. roseus*



Gram positive cocci in Tetrads arrangement - *M.roseus.*



Pink colonies of Micrococcus roseus on Nutrient agar.

PRELIMINARY TESTS

Sl.no	Tests	Results
1.	Gram staining	Gram-Positive
2.	Motility	Non motile
3.	Catalase	Positive
4.	Oxidase	negative

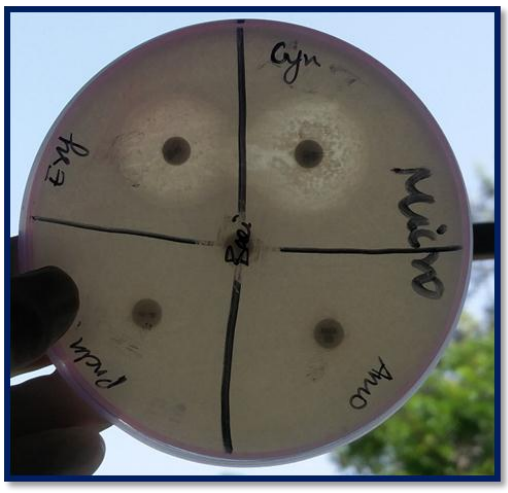
Catalase positive – *M.roseus.*

Colony characteristics of *M.roseus*

S.no	Colony morphology	Inference
1.	Size and colour	1-2 mm and pink colonies
2.	Margin	Entire
3.	Shape	Circular
4.	Opacity	Opaque
5.	Consistency	Smooth
6.	Elevation	Convex

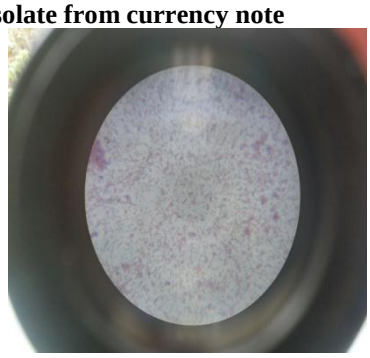
Antibiotic sensitivity test

Micrococcus roseus was found to be highly sensitive to Erythromycin followed by clindamycin. It was found to be resistant to Vancomycin and Pencillin.



Sl.no	Antibiotics	Zone of Inhibition
1.	Amorphicilin	Nil
2.	Erythromycin	23 mm
3.	Clindamycin	25 mm
4.	Penicillin	Nil

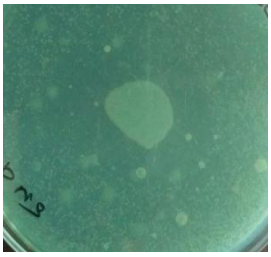
***S.aureus* isolate from currency note**



Gram positive cocci in clusters.

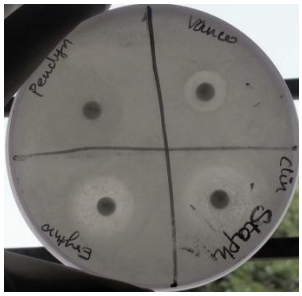
***S.aureus*- Yellow colonies on Mannitol salt agar.**

Coagulase test positive *S.aureus.*



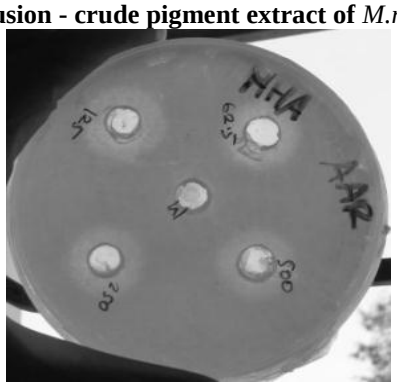
DNAse test- *S.aureus* (isolate from note).

Antibiotic sensitivity test– *S.aureus* (isolate from note).

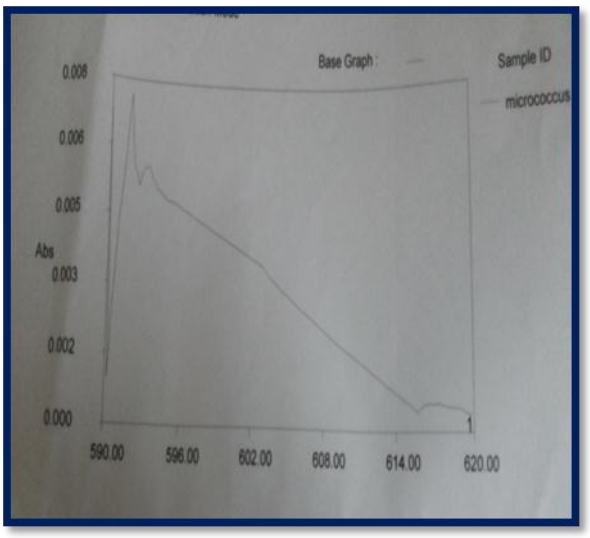


Sl. No.	Antibiotics	Zone of Inhibition
1.	Vancomycin	13mm
2.	Clindamycin	17mm
3.	Erythromycin	25mm
4.	Pencillin	Resistant

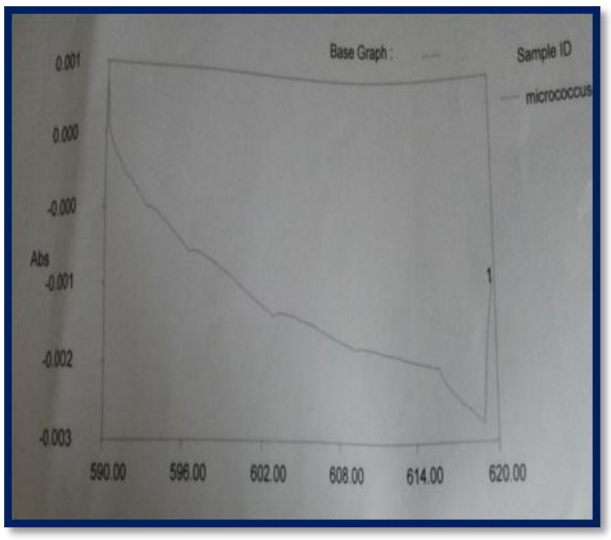
Well diffusion - crude pigment extract of *M.roseus*



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 19 mm at 500, µl concentration 15 mm, at 250 µland found to be resistant at 125 µl and 62.5 µl.



VIS spectrophotometry for *M.roseus.*



UV Spectrophotometry for *M.roseus.*

DISCUSSION

Micro coccus roseus is a Gram positive cocci in tetrads belongs to family Micrococcaceae It is, catalase positive, oxidase positive, produces pink pigment on Nutrient 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. The UV Visible spectro photometric studies showed highest peak indicating estimation of pigment 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 19 mm and 15 mm at 500 µl and 250 µl. The isolate was found to be resistant to pigment extracts at concentration range of 125 µl and 62.5 µl.

SUMMARY AND CONCLUSION

Micro coccus roseus is a Gram positive cocci in tetrads belongs to family Micrococcaceae It is, catalase positive and oxidase positive. The pigments acts as a novel exhibited antimicrobial agents. *Micro coccus roseus* was *sub* cultured and 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 public transport 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 19 mm and 15mm. The current study reported that currency notes plays an important role in spreading infections .It was concluded that Currency note acts as a source of various infections. The pigment extract acts as a novel bio colour against Staphylococcus aureus isolate from currency note. This study was done for the first time to the best of our Knowledge.

ACKNOWLEDGEMENTS

The Authors thank Ms Summera Rafiq, Associate Professor& Head, P.G.Dept of Applied Microbiology for her valuable support and Ms Tharani.V of M.Sc II nd year, Microbiology for extended help in providing culture.

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