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[AJPCR] Article Review Request

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Muhammad Yanis Musdja:

I believe that you would serve as an excellent reviewer of the manuscript, "EXTRACTION AND CHARACTERIZATION OF ANTIBACTERIAL PIGMENT FROM ROSEOMONAS GILARDII YP1 STRAIN IN YERCAUD SOIL," which has been submitted to Asian Journal of Pharmaceutical and Clinical Research. The submission's abstract is inserted below, and I hope that you will consider undertaking this important task for us.

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"EXTRACTION AND CHARACTERIZATION OF ANTIBACTERIAL PIGMENT FROM ROSEOMONAS GILARDII YP1 STRAIN IN YERCAUD SOIL"

Abstract

Objective: The current study focused on antibacterial pigment production from Yercaud hills region soil bacteria.

Methods: In this present study soil sample was collected from coffee plantation in Yercaud, Salem district. The collected soil sample was used to isolate the pigment producing bacterial strains. The pigments were extracted by acidified ethanol and their used to screening the antibacterial activity against clinical pathogens. The potential antibacterial pigment producer was identifying and the pigment was characterized by UV- spec, TLC and FT-IR. And also evaluate the MIC of pigment against clinical pathogens.

Results: The pigment producing bacterial strains were isolated from Yercaud soil, among the isolates YP1 was maximum activity against test pathogens. The potential pigment producer was identified as *Roseomonas gilardii.* The extracted pigments showed the maximum absorbance at 450 nm and their functional groups were identified using FTIR analysis.

Conclusion: From the results, the pigment was extracted from *Roseomonas gilardii* and it may serve as potential antibacterial pigment of interest in the food industries, textile industries in natural colorant and in cosmetic industries.

Dr. Yanis Musdja, M.Sc. <yanis.musdja@uinjkt.ac.id> 14 Desember 2019 21.59
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Dear Editor ajpcr

Thank you very much for your email, I will revise the full paper that you sent

Best regards

Dr. Muhammad Yanis Musdja
[Kutipan teks disembunyikan]

Dr. Yanis Musdja, M.Sc. <yanis.musdja@uinjkt.ac.id>
Kepada: editor ajpcr <ajpcr@innovareacademics.in>
30 Desember 2019 23.07

Dear Editor Asian Journal of Pharmaceutical and Clinical Research (AJPCR)

I have revised the full paper with the title:

EXTRACTION AND CHARACTERIZATION OF ANTIBACTERIAL PIGMENT FROM *ROSEOMONAS GILARDII* YP1STRAIN IN YERCAUD SOIL

Please read my marginal notes, for the improvement of this full paper, as attached (in the form of MS words and pdf)

Thank you for your cooperation.

Best regards
[Kutipan teks disembunyikan]

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EXTRACTION AND CHARACTERIZATION OF ANTIBACTERIAL PIGMENT FROM *ROSEOMONAS GILARDII* YP1STRAIN IN YERCAUD SOIL

ABSTRACT

Objective: The current study focused on antibacterial pigment production from Yercaud hills region soil bacteria.

Methods: In this present study soil sample was collected from coffee plantation in Yercaud, Salem district. The collected soil sample was used to isolate the pigment producing bacterial strains. The pigments were extracted by acidified ethanol and their used to screening the antibacterial activity against clinical pathogens. The potential antibacterial pigment producer was identifying and the pigment was characterized by UV- spec, TLC and FT-IR. And also evaluate the MIC of pigment against clinical pathogens.

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Conclusion: From the results, the pigment was extracted from *Roseomonas gilardii*and it may serve as potential antibacterial pigment of interest in the food industries, textile industries in natural colorant and in cosmetic industries.

Keywords: Pigment, *Roseomonas gilardii,* Extraction, Antibacterial activity, Characterization

Commented [MYM1]: Objective: The current study focused on extraction and characterization of antibacterial pigment from roseomonas gilardii yp1 strain in Yercaud hills region

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INTRODUCTION

Pigment are the colors that we observe at each steps of our lives, because present in the entire organism in the world, where plants are the principal producers. Pigments are present in leaves, fruits vegetable and flowers; they are also found in skin, eyes, and other animal structures; and in bacteria and fungi. Pigments have a well-known pharmacological activity such as anticancer and effective against cardiovascular diseases [1,2].Through many natural colors are available from ores, insects plants and microbes; microbial colorants play a significant role is food coloring agent, because of its production and easy down streaming process[3,4].There are number of natural pigments but only a few are available quantities for industrial production. Production of pigments from microorganism is beneficial over other sources because microorganisms can grow rapidly which may lead a high productivity of the product [5]. Certain bacteria produce water soluble pigments which spread through the medium in which they grow; other give pigments are

soluble in fat. The yellow pigment from zeaxanthin from *Flavobacterium* species can be used as addictive in poultry feed to fortify the yellow color of the skin of birds or to accentuate the colors of the yolk of the egg. A yellow pigment zeaxanthin from *Flavobacterium* sp can also be used in cosmetic and in food industry. Canthazanthin from the photosynthetic bacterium *Bradyrizobium* sp has been used in fishfeed and for numerous years. *Halobacterium* is also one more source of canthaxanthin, astaxanthin from *Agrobacterium aurantiacum* [6].The production and application of microbial pigment as natural colorants has been studied by various researchers and is one of the emerging fields of research [7]. Efforts have been made in order to reduce production costs of microbial derived pigments compared to those of synthetic pigments or pigments extracted from natural sources. Innovations will progress the economy of pigments production by isolating new or creating better microorganisms, by improving the process. Hence, work on the microbial bacterial pigments should be excelled especially in finding cheap and suitable growth mediums which can reduce the cost and increase its applicability for industrial production [5]. This present investigation has been under taken to study the antimicrobial activity of microbial pigment isolated from coffee rhizosphere soil sample against clinical pathogens.

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MATERIAL AND METHODS

Collection of sample

The soil sample were collected from coffee plant rhizosphere area in Yercaud, Tamil Nadu from these samples antibacterial pigment producing bacteria was isolated and the bacterial cultures were used for the present study.

Commented [MYM5]: Tamil Nadu, India

Isolation of pigment producing bacterial strains

Collected soil sample were used for serial dilution upto $10^{-1}$to 10^{-7} dilutions were plated on nutrient agar plates and the plates were kept for incubation at 37°C for 48hours. The nutrient agar plates were observed for growth after the incubation.

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Extraction of pigment from pigment producing bacteria

The pigment producing bacterial strains were harvested by centrifugation at 6000 rpm for 10 minutes. Then the supernatants were discarded and the pellets were re-suspended in acidified ethanol (4ml of 1M HCL in 96 ml ethanol). Then the mixture was vortex and the suspension was centrifuged at 6000 rpm for 10 minutes and the supernatant was collected. The centrifugation was repeated till the pellet changes to colorless. The crude pigments were allowedto acidified ethanol

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evaporation. After evaporation of acidified ethanol, dry pigment was collected for further uses [8].

Screening of antibacterial pigment producing bacteria

An overnight log culture of each pathogenic strain likeE. *coli, Klebsiella* sp, *Enterococcus* sp, *Pseudomonas* sp and*Proteus* sp. was spread evenly on a Muller Hinton Agar(MHA) plate by swab. Wells were made on the MHA plates by using a gel puncture. After that the crude pigment extract (100 µl) was added into each well, and the plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measuring the diameter of the zone formed around each well.

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Identification of potential pigment producing bacteria

Colony characterization of pigment producing bacteria was done by, based on its size, shape, color, margins, opacity, consistency, elevation, Gram staining and motility. The biochemical per found were indole, methyl red, voges proskauer, citrate utilization, urease, oxidase, catalase and triple sugar iron test. The pure culture of isolate was maintained on nutrient agar slant for further investigation.

Commented [MYM10]: Characterization of pigment-producing bacterial colonies has been carried out based on: size, shape, color, margins, opacity, consistency, height, Gram staining and motility. The biochemistry per discovered was indole, methyl red, voges proskauer, utilization of citrate, urease, oxidase, catalase, and three-sugar iron test. Pure culture of isolates was maintained on nutrient agar sloping for further investigation

Characterization of extracted bacterial pigment

UV-spectroscopy analysis of crude pigment

UV– spectrum scanning of the obtained bacterial pigment was performed in a solution of Tris HCl 50 mM (pH 8.5) to a concentration of 1 mg/ml in the wavelength range of 350-750nm to find out the maximum absorption spectra and Tris HCl 50 mM (pH 8.5) as blank. The spectra were determined using UV-visible spectrophotometer [9].

Commented [MYM11]: Scanning with UV-visible spectrophotometer for bacterial pigments obtained was carried out in a 50 mM Tris HCl solution (pH 8.5) up to a concentration of 1 mg / ml in the range of wavelengths 350-750 nm to determine the maximum absorption spectrum and Tris HCl 50 mM (pH 8.5) blank.

Thin layer chromatography (TLC) analysis

The purified pigment was analyzed by thin layer chromatography with silica gel. The solvent system consists of chloroform: methanol (9:1; v/v). The chromatography chamber with the solvent was kept for 20 minutes for the equilibration. The sample was spotted on the silica gel sheet using a capillary tube and air dried. The TLC sheet was then dipped in the solvent system.

After 45 minutes the TLC sheet was carefully removed and the Retention factor (Rf) value was calculated according to the following equation from the chromatogram [10].

Commented [MYM12]: Purified pigments were analyzed by silica gel thin layer chromatography. The ratio of chloroform: methanol solvent used was (9: 1; v / v). Chromatographic chambers with solvents were stored for 20 minutes for equilibrium. The sample was placed on a silica gel sheet using capillary tubes and dried with air. Then the TLC sheets were dipped into the solvent system. After 45 minutes, the TLC sheets were carefully removed and the Retention factor (Rf) value was calculated according to the following equation from the chromatogram

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

Fourier transform-infrared spectroscopy (FT-IR) analysis

The pigment was analyzed using FT-IR spectroscopy. The crude bacterial pigmentwas encapsulated in KBr at a ratio of 1:100. The IR spectra were collected using a Shimadzu spectrometer within the range of 400-4000cm^{-1}. The FT-IR spectroscopy is used to analyze the functional group of the extracted pigments.

Commented [MYM13]: Pigments were analyzed by using FT-IR spectroscopy. The crude bacterial pigments were encapsulated in KBr at a ratio of 1: 100. The IR spectrum was scanned by using a Shimadzu spectrometer in the range of 400-4000cm-1. FT-IR spectroscopy obtained was used to analyze the functional groups of extracted pigments.

Minimum inhibitory concentration (MIC) of bacterial pigment

The clinical pathogenic (*E.coli, Klebsiella* sp, *Staphylococcus aureus, Pseudomonas* sp, *Proteus* sp and*Enterococcus*sp) cultures were inoculated into the nutrient broth and incubated for overnight. The MHA medium was prepared and the petriplates were sterilized. Then sterilized medium was poured into the plates and allowed them to solidify. The clinical pathogens were swabbed onto the plates. Well were cut in the agar plates by using well puncture. The crude extract was dissolved in acidified ethanol and 20µl, 40µl, 60µl, 80µl and 100 µl were added to the well and incubated these plates at 37ºC for 24 hrs.

Commented [MYM14]: Petri plates

RESULTS

Isolation of pigment producing bacteria

The collected coffee rhizosphere soil samples were subjected to spread plate method on nutrient agar plates. The bacterial cultures were selected from based on the different colony morphology. Totally 30 bacterial strains(YP1 to YP30) were isolated from soil sample (Fig.1). Among the 30 bacterial isolates, four isolates (YP1, YP3, YP5 and YP27) were producing pigments and the pigmented bacterial colonies were used to further screening process.

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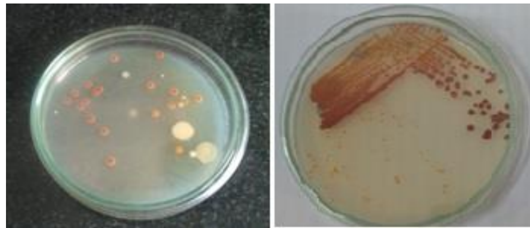

Fig.1: Isolation of pigment producing bacteria

Screening of antibacterial pigment producing bacteria

The potential pigment producing four bacterial isolates were centrifuged and collected the cell free supernatants were used to screening as a crude pigment. Among the four isolates the bacterial isolate YP1 was highly inhibition effect of clinical pathogens (Table. 1). The highest activity producing YP1 bacterial strain was used as further analysis.

Commented [MYM16]: Potential pigments that produce four bacterial isolates were centrifuged and collected cell-free supernatants were used for screening as crude pigments. Among the four isolates, YP1 bacterial isolate had a very high inhibitory effect on pathogenic bacteria (Table 1). The highest activity producing YP1 bacterial strains was used as further analysis.

Table.1: Antibacterial activity of pigment producing bacterial isolates

Bacterial crude extract	**Zone of inhibition (mm)**				
	Klebsiella sp	*Enterococcus* sp	*Pseudomonas* sp	*E. coli*	*Proteus* sp
YP1	42	41	35	39	38
YP3	21	25	29	20	23
YP5	21	32	29	17	24
YP27	24	-	34	19	21

Identification of the potential pigment producing bacterial isolate

The bioactive pigment producing potential bacterial isolate was then preliminary characterized,according to Bergey's manual of determinative bacteriology based on morphological and biochemical characterization. The antibacterial pigment producing bacterial isolate was translucent, smooth colonies of about 1 mm diameter with profuse brown pigmentations was selected for further studies. The biochemical characterization of the potential bacterial isolate was tabulated in Table 2. The isolated potential bacterial strain was identified as *Roseomonas gilardii*by 16S rRNA sequencing. The isolated antibacterial pigment producing bacterial isolate

Commented [MYM17]: Potential bacterial isolates that produce bioactive pigments were

Commented [MYM18]: Bacteria isolates that produce translucent antibacterial pigments

YP1 as showed the 98% similarity with *Roseomonas gilardii*and their genbank accession number is MH324463 (Fig.2).

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Table.2: Biochemical characterization of potential isolate

S.No.	Characterization	Strain YP1
1	Gram staining	Gram negative cocci
2	Catalase test	Positive
3	Oxidase	Negative
4	Indole	Positive
5	Methyl red	Negative
6	Vogesproskaur	Negative
7	Citrate utilization test	Positive
8	Urease test	Positive
9	TSI	Negative

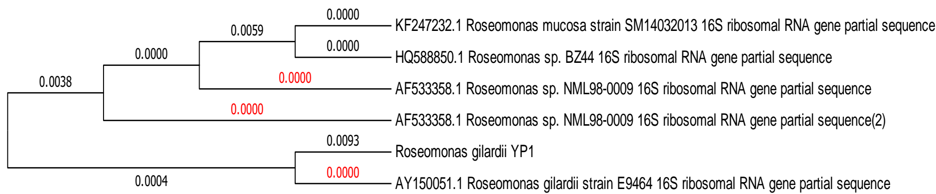


Fig .2: Phylogenetic analysis of bacterial isolate

Extraction of bacterial pigment

The antibacterial pigments were extracted from YP1 pigment producing bacterial strain. And their extracted pigment (Fig.3) was characterized for further analysis.

Fig.3: Extraction of potential bacterial pigment

UV- Vis spectroscopy analysis of crude pigment

Absorption spectra of pigments from the YP1 strain was studied in the visible range between the wave length of 350 to 750nm and spectrophotometric analysis at the respective wave length at which maximum absorbance (λ_{max}) were observed in 450 nm (Fig.4).

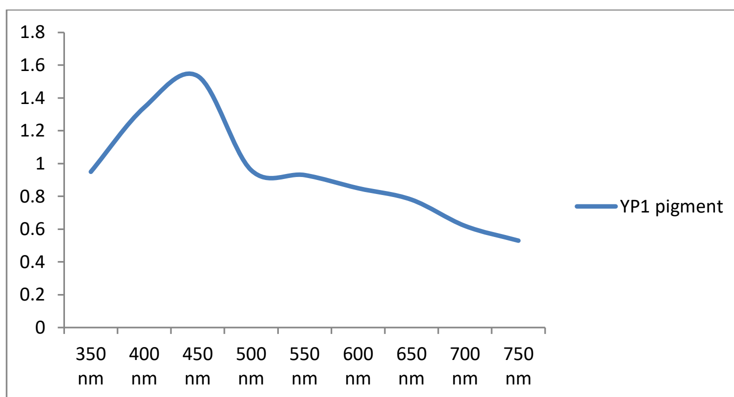


Fig.4: UV-spectra absorption of YP1 strain

TLC analysis of crude pigment

The extracted pigment was separated by thin layer chromatography with silica coated TLC plate. The solvent system chloroform: methanol (9:1) was used for separation of pigment. TLC showed the presence of a pigment which migrated as brown component in the sheet with retention factor **0.82**.

Commented [MYM20]: The ratio of chloroform: methanol (9: 1) solvent was used for pigment separation

FT-IRanalysis of crude pigment

The extracted crude pigment was characterized by FT-IR spectrum. The pigment obtained from the carboxylic acids, alkenes, phenols, alkanes and primary amines in the samples based on the spectra (Fig.5).

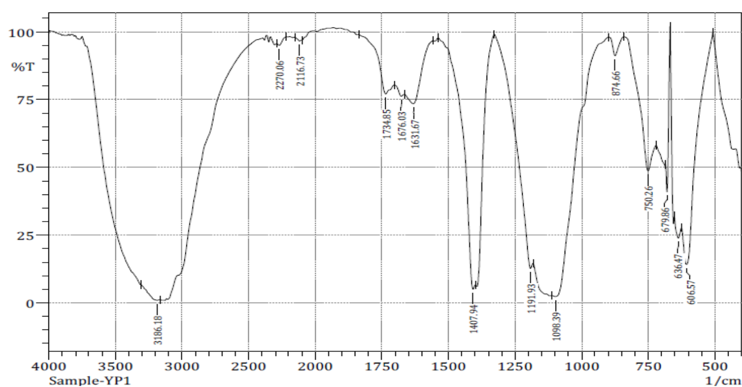


Fig. 5: FT-IR characterization of pigment

Minimal inhibitory concentration of pigment against clinical pathogens

The pigment was tested for antibacterial activity against six selected clinical pathogens like, *Staphylococcus aureus*, *Klebsiella*sp, *Enterococcus*, *E.coli*, *Pseudomonas*sp *and Proteus*sp by well diffusion method. The maximum zone of inhibition observed in *E. coli* bacterial strain (Table.3 and Fig.6).

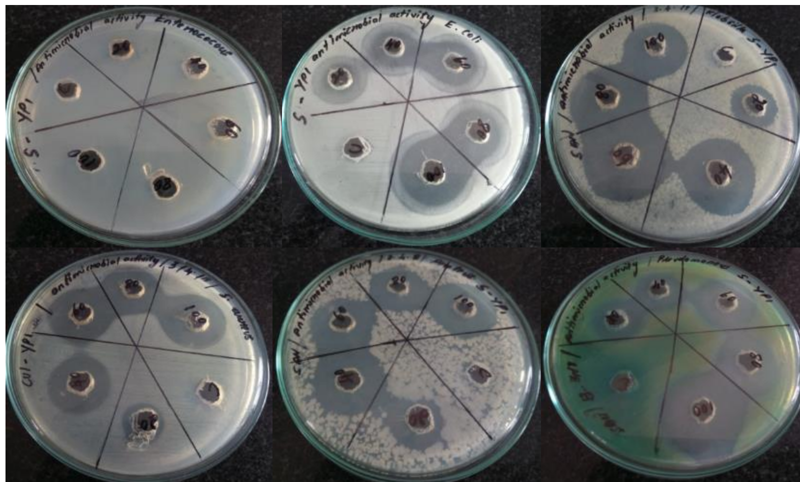
Commented [MYM21]: The pigment was tested for antibacterial activity against six selected bacterial pathogens namely:

Table.3: MIC of bacterial crude pigment

Test pathogen	**Concentration of crude pigment (mg/ml)**				
	20µl	**40µl**	**60µl**	**80µl**	**100µl**
E. coli	21mm	26mm	27mm	28mm	30mm
*Pseudomonas*sp	21mm	21mm	26mm	27mm	28mm
Staphylococcus aureus	14mm	21mm	23mm	23mm	25mm

Enterococcus sp	-	-	-	-	-
*Klebsiella*sp	16mm	23mm	25mm	25mm	26mm
*Proteus*sp	16mm	23mm	25mm	25mm	26mm

Fig .6:MIC Concentration of YP1 bacterial pigment



DISCUSSION

Totally 30 pigmented bacterial strains were isolated from coffee plant rhizosphere soil sample in Yercaud hills region, Tamil Nadu. The isolated colonies were following colors: brown, green, dark orange, orange, red, yellow, pink, light brown, pale yellow, dark greenish yellow. Among the 30 bacterial strains only 4 bacterial strains producing antibacterial pigments. However the brown pigment producing colonies were found predominant. Earlier studies in 15 pigment producing bacteria were isolated from 8 soil samples collected from different places of Dhaka city. The isolated colonies were of following colors: red, brown, pink, black, violet, blue, green, cream, golden, dark orange and light yellow. Among those the yellow was most dominant [11,12]. Previous reported the soil and water samples collected from different parts of Pune were used for isolation of pigment producing bacteria. Five pigment producing bacteria were identified and characterized which were red, yellow, cream, light orange in color [8].The present study report that the isolates purified by re-streaking further on nutrient agar incubated for 48 hours at 37ºC. Following overnight incubation in nutrient agar at 37ºC, the isolates were preserved in 30% glycerol at -20ºC. The potential antibacterial pigment producer was then characterized by Bergey's

Manual of determinative bacteriology based on morphological and biochemical characterization. The molecular identification of the potential bacterial isolate YP1 was identified as *Roseomonas gilardii*by 16S rRNA sequencing. In earlier reported the pigment producers were then identified according to Bergey's manual of determinative bacteriology [13] based on Gram staining, biochemical characteristics and growth pattern on selective and differential media. The strains were found to belong to the genera *Aeromonas* (20%), *Pseudomonas* (20%), *Chromobacterium* (13.3%), *Flavobacterium* (6.7%), *Bacillus* (13.3%), *Xanthomonas* (6.7%) and *Escherichia* (20%) [11]. A taxonomic identification of the test isolates under study was reported by performing Gram's test, studying cultural characteristics of the isolates on selective and differential media and biochemical tests based on the Bergey"s Manual of Determinative Bacteriology. Biochemical tests performed were IMViC, oxidase and catalase tests. Samyuktha and Sayali Naphade Mahajan were identified the pigment producing bacteria was carried out the 3 isolates were found to be Gram negative coccobacilli [14,15]. The intracellular antibacterial pigment was extracted by various method were used centrifugation and addition of acidified ethanol so that the cell gets lysed and intracellular pigment can be extracted. The extracted pigment was showed in brown color. Vora *et al.,* were extracted the pigment by different solvents with their different concentrations, they are acetone, ethyl acetate, chloroform and methanol were used. There was no pigment extraction observed in chloroform and ethyl acetate solvent followed by acetone and methanol are the solvents, which have capacity to extract the pigment from the bacterial cell. But the highest extraction of pigment was shown in methanol and the combinations of acetone: methanol (2:1) solvent system showed the maximum extraction (85%) of pigment [10].In a marineisolate *Vibrio* sp. pigment was hydrophobic nature so that maximum extraction of the pigment was extracted by using different solvents such as ethanol, methanol, chloroform, ethyl acetate, petroleum ether, acetoneand distilled water also have been considered to find the suitable solvent for effective extraction [16]. The Yercaud region isolates of the four pigment (YP1, YP2, YP5, YP27) extracted from isolates showing clear zone inhibition against *Klebsiella* sp (42mm), *Enterococcus* sp (41mm), *Pseudomonas* sp (35), E. coli (39) for YP1 strain. *Klebsiella* sp (21mm), *Enterococcus* sp (25 mm), *Pseudomonas* (29mm), *E. coli* (20 mm) for YP2, *Klebsiella*sp (21 mm), *Enterococcus*sp (32 mm), *Pseudomonas*sp (32 mm), *E. coli* (17 mm), *Proteus* sp (24 mm) for YP5 strain, *Klebsiella* sp (24mm), *Enterococcus* sp (resistance), *Pseudomonas* sp (34mm), *E. coli* (19 mm), *Proteus* sp (21mm) more zone of inhibitory was observed in YP1 strain when compared to

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other strains for all human pathogen. In earlier reported the prodigiosin pigment was extracted from isolate showed the clear inhibition zone against *Bacillus cereus* (12mm), *Staphylococcus aureus* (7 mm), *Escherichia coli* (6 mm) [10]. In another report showed the prodigiosin pigment activity against *Staphylococcus aureus* (17.5 mm), *Bacillus cereus* (10.5 mm), *Escherichia coli* (resistance), but this report showed the antibacterial activity of prodigiosin pigment against *B. cereus* was higher than compared with *E. coli*[17].The UV-visible absorption spectrum of antibacterial pigment from the YP1 strain was studied in the visible range of the wave length of 350-750nm and spectrophotometric analysis at the respective wavelength which maximum absorbance was observed in 450 nm. In earlier *Vibrio* sp producing pigment was observed in maximum spectrum was 536 nm in UV-vis spectrometry [16]. Similarly the prodigiosin pigment from *Serratia* sp was showed the strong absorbance in the UV region [10,18].The extracted pigment was separated by thin-layer chromatography with the solvent system used for chloroform: methanol (9:1). TLC showed the presence of a pigment was which migrated as brown component in the sheet with retention factor as 0.82. The purified pigment was analyzed by thin-layer chromatography with silica gel G-60. The solvent system consists of chloroform: methanol (95:5; v/v). The chromatography chamber with the solvent was kept for 20 min [10].

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Commented [MYM28]: The extracted pigment was separated by thin layer chromatography using a chloroform: methanol solvent in the ratio (9: 1).

Commented [MYM29]: Purified pigments were analyzed by thin layer chromatography with silica gel G-60 using a chloroform: methanol solvent with a ratio (95: 5; v / v).

CONCLUSION

The polyphenolic rich coffee plant rhizosphere constitutes an the study has extremophilic condition in addition to the lower atmospheric temperature prevailing in Yercauds that greatly influences bio-geo nutrient recycling by retarding. Extremophiles have the tendency to acquire potential characteristics for their survival in competitive environment. Hence the presence study focused on pigment producing bacteria from such an extremophilic condition and found a higher pigment producing activity with potential antimicrobial characteristic in the isolates. This study thus attempted to explore the extremophillic microbial ecology for pigment production with its antimicrobial characteristics.The identified isolates from soil were found to the YP1 pigment strain, able to produce pigment. In this regard, this study is an initiative approach towards the use of bio-colorants which find its applications in numerous sectors as an alternative for synthetic chemicals. To conclude, microbial pigment production is one of the emerging fields of research to demonstrate its potential for various industrial applications.

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Conclusions must be rewritten in total, because it is too long and do not describe the research title and research objectives.

REFERENCE

[1] Daniela Cristea, Gerard Vilarem. Improving light fastness of natural dyes on cotton yarn. Dyes and Pigments 2006; 70(3): 238-245.

[2] Delgado Vargas F, Jimenez AR, Paredes Lopez O. Natural pigmets: carotenoids, anthocyanins and betalains- characteristics, biosynthesis, processing and stability. Crit Rev Food Sci Nutr 2000; 40(3): 173-289.

[3] Bechtold T, Mussak R. Handbook of natural colorants; A John Wiley and Sons, Ltd., Publication 2009.

[4] Galaffu N, Bortlik K, Michel M. An industry perspective on natural food colour stability. Colour additives for foods and beverages. Woodhead Publ Ser Food Sci Tchnol Nutr 2015; 91-130.

[5]Venil CK, Zakaria ZA, Ahmad WA. Bacterial pigments and their applications. Process Biochem 2013; 48: 1065-1079.

[6] Dufosse L, Galaup P, Yaron A, Arad SM. Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality. Trends in Food Science and Technology 2013; 16(9): 389-406.

[7] Venil CK, Lakshmanaperumalsamy P. An insightful overview on microbial pigment, prodigiosin.Electronic Journal of Biology 2009; 5: 49-61.

[8] Sinha S, Choubey S, Ajay Kumar A, Bhosale P. Identification, characterization of pigment producing bacteria from soil and water and testing of antimicrobial activity of bacterial pigments.Inst J Pharm Sci Rev Res 2017; 42(2): 119-124.

[9] Zerrad A, Anissi J, Ghanam J, Sendide K, Hassouni M.Antioxidant and antimicrobial activities of melanin produced by a *Pseudomonas balearica* strain.Journal of Biotechnology Letter 2014; 5(1): 87-94.

[10] Vora JU, Jain NK, Modi HA.Extraction, characterization and application studies of red pigment of halophile *Serratia marcescens* KH1R-KM035849 isolated from Kharaghoda soil. International Journal of Pure and Applied Bioscience 2014; 2(6): 160-16.

[11] Md. Mamunur Rashid, Md. Fakruddin, Reaz Mohammad Mazumdar Fatema Kaniz, Md. Alimuddin Chowdury, Anti-bacterial activity of pigments isolated from pigment forming soil bacteria.British Journal of Pharmaceutical Research2014; 4(8): 880-894.

[12] Palaniswamy M, Vishnu TS. Isolation and identification of *Chromobacterium* sp. from different ecosystems. Asian Journal of Pharmaceutical and Clinical Research 2016; 9(9) 253-257.

[13] Holt JG. Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore 1994.

[14] S. Samyuktha and Sayali Naphade Mahajan, Isolation and identification of pigment producing bacteria and characterization of extracted pigments, *International Journal of Applied Research* 2(7): 657-664 (2016).

[15] Shubhangi A Rajguru, Neha Sawant, Akshayaveni Valmiki, Padma V Deshmukh. Isoaltion and identification of pigment producing bacterial isolates from different terrestrial habitats in Thane District, M.S, India.World Journal of Pharmacy and Pharmaceutical Science (2015); 5 (1): 618-628.

[16] Shiva Krishna Pabba, KrishnaG, RS Prakasha RS, Singara Charya MA. Antibacterial activity of textile fabrics treated with red pigments from marine bacteria.Journal of Marine Biosciences 2015; 1(1): 11-19.

[17] Chandni Gulani, Sourav Bhattacharya, Arijit Das. Assessment of process parameters influencing the enhanced production of prodigiosin from Serratia marcescens and evaluation of its antimicrobial, antioxidant and dyeing potentials. Malaysian Journal of Microbiology 2012; 8(2): 116-122.

[18] Nora M Elkenawya, Aymen S Yassinb, Hala N Elhifnawya, Magdy A Aminb. Optimization of prodigiosin production by Serratia marcescens using crude glycerol and enhancing production using gamma radiation. Biotechnology Reports 2017; 14: 47-53.