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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF PIGMENTS FROM *Staphylococcus sp*. AND *Rhodotorula sp.*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Microbial pigments offer promising avenues for various applications due to their better biodegradability and higher compatibility with the environmentt. Microbial pigments have numerous therapeutic properties like anticancer, antiproliferation and immunosuppression. Pigment producing probiotic microbes were isolated from curd and rice water. The isolated pigment producing *Rhodotorula sp*. and *Staphylococcus sp*. were characterised and evaluated for their antibacterial activity by disc diffusion assay and antioxidant activity by DPPH methods. Based on the morphological, biochemical and molecular characterisation, the unknown orange pigment producer was identified as *Rhodotorula sp*., while yellow pigment producer was identified and confirmed as *Staphylococcus sp.* Both the isolates demonstrated antibacterial and antioxidant activities, suggesting the therapeutic efficiency of microbial pigments.

Keywords: Pigments; antibacterial; antioxidant; *Staphylococcus sp*. and *Rhodotorula sp.*

1. INTRODUCTION

The emergence of drug resistance is related to the overuse of present antibiotics [1]. In order to overcome the emergent drug resistance, novel antibiotics from new sources are in demand. The demand for new drugs expand with the increased spread of new pathogens and their resistance to antibiotics. Products of microbes isolated from novel ecosystem can be a potential alternative source for new drugs. Previous studies have reported the screening of pigments isolated from various soil microbes [2]. Natural pigments obtained from insects, plants and animals were the colorants used since prehistoric period [3]. Microbial pigments are actually coloured secondary metabolites secreted during stress conditions. These pigments have diverse range of applications in various pharmaceutical, food and textile industries. Many microorganisms, including bacteria, fungi, yeast and moulds are employed for the industrial production of various pigments through fermentation technology. Microbial pigments aid in protection from UV rays, act as antioxidants, protect from extreme heat and cold and also function as antimicrobials and anticancer agents. Due to its potential activity against many human pathogenic bacterial strains, pigments can be used for treatment of patients infected with such bacterial pathogens. Microbial pigments also showed strong antioxidant activity helpful in scavenging of free radicals. In the food industry, the pigments are used as additives, colour intensifiers, antioxidants etc. [4]. Pigments like

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indigoids, anthraquinones and naphthoquinones will hold potential applications in food industry in near future [5]. Despite therapeutic applications, bacterial pigments also possess applications in food and textile industries. In food and textile industries, these pigments are used as colorants. Further research is required to establish the therapeutic potential of the pigments from microbes. There is an increasing interest involving microbial pigments as a possible alternate source of therapy for infections by drug resistant bacteria and other diseases. In this direction, microbial pigments may open up new avenues in biotechnology in near future. Hence, the current work was undertaken to evaluate the antibacterial and antioxidant potential of pigments obtained from a bacterium and yeast.

2. MATERIALS AND METHODS

2.1 Isolation of Pigment Producing Microbes-Sample Collection

Homemade curd and rice water samples were used for isolation of pigment producers.

2.2 Isolation of Pigment Producing Bacteria from Rice Water and Curd

The curd and rice water samples were collected in sterilized falcon tubes and brought to the laboratory.1ml of collected samples were subjected to serial dilution. 100 µl of 10^{-5} dilution of each sample was spread on NB agar medium (pour plate method) and incubated at 37°C for 3 days. All the pigment producing single cell colonies that appeared on the agar plates were picked and successively streaked on NB agar plate.

2.3 Purification and Selection of Pigment Producing Bacterial Isolates

The isolated colonies were purified by three successive streaking on NB agar medium. Potential isolates were selected based on the difference in the colour of colonies. For this, isolates were streaked on Nutrient agar plates and incubated at 37°C for 48 h and observed for pigment production. The isolates that showed bright pigmentation were selected. Purified isolates were preserved as agar slants.

2.4 Gram's Staining

Gram's staining was performed for the identification of the gram reaction of the bacteria following the standard procedure [6].

2.5 Production of Microbial Pigments

Pigment producers were grown in nutrient broth and nutrient agar medium. For large scale production of pigments, equal number of cells were inoculated in 150 ml freshly prepared nutrient broth and incubated in an incubator shaker at temperature of 30°C at 200 rpm for 48 hours and visually observed for pigment production.

2.6 Extraction of Microbial Pigments

Pigments producing isolates were grown in 150 ml freshly prepared NB at 30°C in an incubator shaker at 200 rpm for 48 h. After 48 h, bacterial culture was centrifuged at 7500 rpm for 20 min. After centrifugation, supernatant was discarded and bacterial cell pellets were processed for extraction of pigments. To the cell pellets, 1ml mixture of acetone and methanol (3:1) was added and vortexed until the cell pellets turned colourless. The cell debris was then discarded, while the supernatant was transferred to a glass petriplate and dried overnight in an incubator at 37°C. Dried pigments were scrapped out and dissolved in methanol at a concentration of 1 mg/ml [7].

2.7 Antimicrobial Activity of Crude Microbial Pigments

To screen for antimicrobial activity, crude pigments were tested against a few bacteria (gram positive and gram negative) by disc diffusion method. The appearance of zones of inhibition is indicative of antimicrobial activity of crude pigments against the test organism.

2.8 Antioxidant Activity by DPPH Radical Scavenging Activity

The ability of pigments from the microbes to scavenge 1, 1- diphenyl-2 picrylhydrazyl (DPPH) was measured by the reported method [8]. A mixture of absolute methanol served as blank. Ascorbic acid was used as standard and different concentrations of the extract (100,200,300,400 and 500 µg/ml) were marked as tests. Finally, DPPH reagent was added to all the test tubes including blank. Then, the absorbance of all samples was read at 517nm.

2.8.1 Calculation

% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

3. RESULTS AND DISCUSSION

3.1 Isolation of Pigment Producing Bacteria

Both curd and rice water samples were plated on nutrient agar plates. Most of the colonies that appeared after 24hrs were cream white, while few yellow colonies and orange-coloured colonies were observed on third day of incubation at 37°C (Fig. 2). Both the yellow- and orange-coloured colonies isolated from rice water and curd respectively were picked for further study.

3.2 Purification of Pigment Producing Bacterial Isolates

Two pigment producing bacterial isolates (orange coloured and yellow coloured), were picked and successively purified by streaking three times on NB agar medium. During incubation, mild pigmentation was observed after 2 days and more intense pigmentation was observed on 3rd day (Fig. 1 & 2). The purified bacterial cultures were stored as agar slants.

3.3 Gram Staining

On performing Gram staining, the yellow-coloured isolate was found to be Gram positive cocci in clusters (Fig. 3a), suggesting the possibility of *Staphylococcus sp*. The other isolate whose pigmentation was orange revealed globose and elongated buds, suggesting the isolate to belong to the group of yeasts (Fig. 3b).

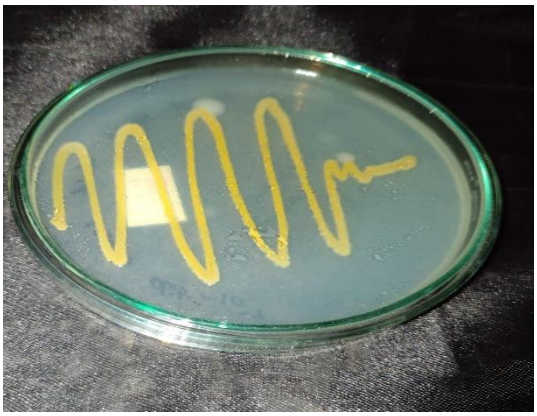

Fig. 1. Isolation of pigment producing *Staphylococcus sp.*

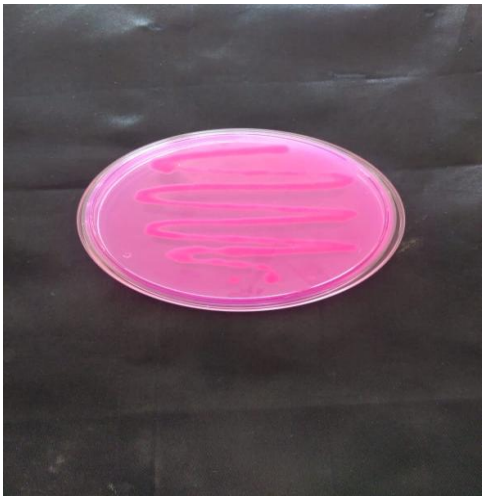

Fig. 2. Isolation of pigment producing *Rhodotorula sp.*

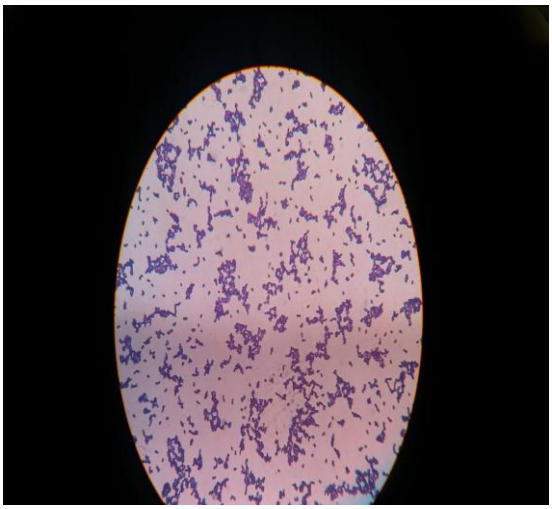

Fig. 3a. Gram positive cocci in clusters

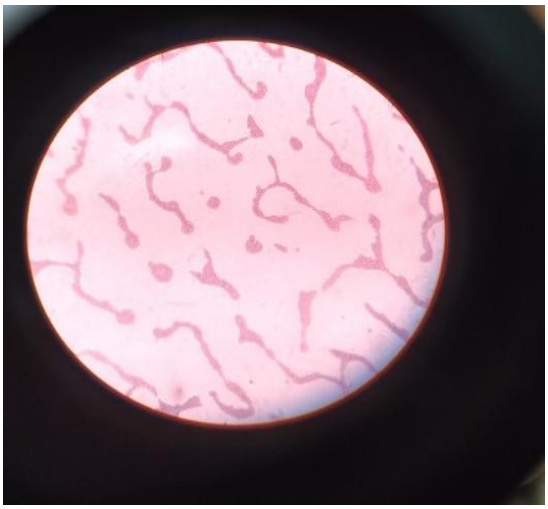

Fig. 3b. Globose buds in Gram staining

3.4 Large Scale Production of Microbial Pigments in Liquid Culture

Both the isolates showed pigment production when streaked on nutrient agar medium or in nutrient broth. We further tested the pigment production in large scale in liquid medium. Both the isolates were inoculated in liquid medium and incubated in an incubator shaker at 30°C at 200 rpm for 48 h. As expected after incubation, the isolates produced pigments, orange and yellow.

3.5 Antibacterial Activity

The pigments were isolated and their role against a few bacterial isolates were checked by disc diffusion assay. The diameter of the zones was measured and results were tabulated (Tables 1& 2).

3.6 Extraction of Pigments

Crude microbial pigments were extracted using methanol from freshly grown bacterial culture. After extraction, orange-coloured pigments and light-yellow coloured pigments were observed in the medium.

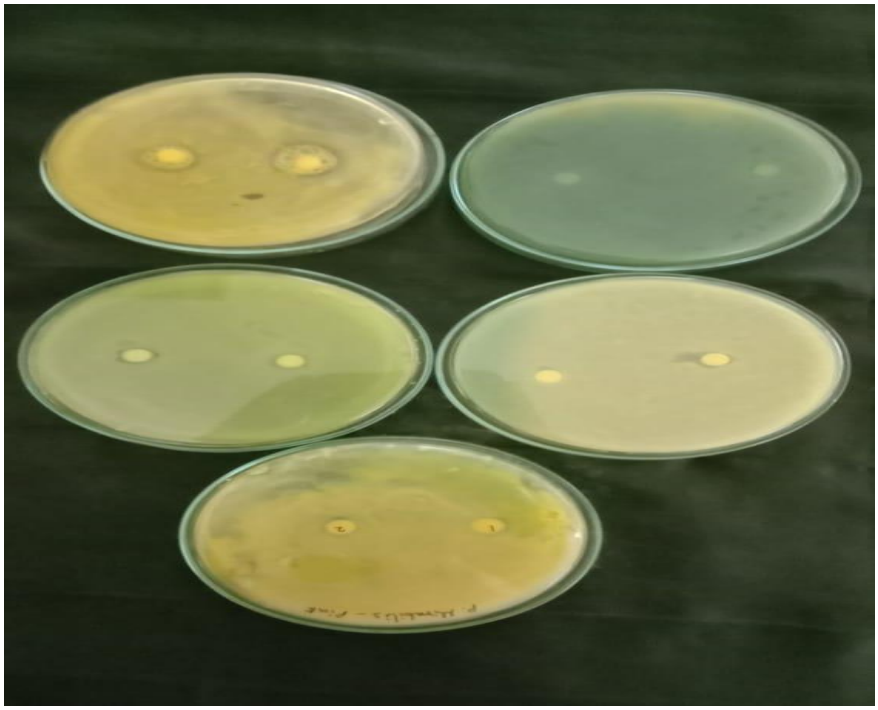

Fig. 4. Plates showing zones of inhibition

Table 1. Antibacterial activity of pigments from *Staphylococcus sp.*against a few bacterial sp.

Serial No.	Bacterial sp.	Zones of Inhibition (Dia in mm)
1.	*Escherichia coli*	8 mm
2.	*Proteus mirabilis*	8 mm
3.	*Pseudomonas aeruginosa*	9 mm
4.	*Klebsiella pneumonia*	8 mm
5.	*Staphylococcus aureus*	11mm

Table 2. Antibacterial activity of pigments from *Rhodoturula sp*.against a few bacterial sp.

Serial No.	Bacterial sp.	Zones of Inhibition (Dia in mm)
1.	*Escherichia coli*	16 mm
2.	*Proteus mirabilis*	8 mm
3.	*Pseudomonas aeruginosa*	13 mm
4.	*Klebsiella pneumonia*	10 mm
5.	*Staphylococcus aureus*	8 mm

Microorganisms are known to produce a variety of pigments; therefore, they are promising source of food colorants and pharmaceuticals. Keeping in mind the applications of microbial pigments in food and pharmaceutical industry, this study was undertaken to identify the pigment producing microbes and the characterization of pigments. Pigments produced by all the isolates were further characterized. Pigments were extracted using acetone and methanol (3:1) solvent mixture. Microbial pigments contain biologically active compounds which possess properties like antioxidant, antitumor and anti-inflammatory. Based on the study, it is concluded that the purified crude microbial pigments can be used in various industries such as food, pharmaceuticals and cosmetics with commercial value. The antibacterial activity of Rhodotorula sp. was more prominent than pigments from Staphylococcus sp. The zone size of Staphyloccoccal pigments ranged between 8mm and 9 mm in diameter, while the zone size was higher in pigments from Rhodotorula sp, ranging between 8mm & 16mm. A good antibacterial activity by *Rhodotorula* sp. was seen against *E.coli*, whose zone size was 16 mm. The antioxidant activity of the pigments from *Staphylococcus* sp & Rhodotorula sp. was observed as percentage of inhibition and was observed to be 60% & 53% respectively. Thus, the pigments isolated were found to possess both antibacterial & antioxidant activity. Recently, a review has been published on the biosynthesis of these compounds from bacteria [9]. With the passage of time, the investigation of new, vital and bioactive compounds from bacterial sources have increased as compared to other sources. Anthocyanin is reported to possess diverse biological activities which has positive influence on health and reduce the risk of cancer [10-12]. Anthocyanin is also known to possess anti-inflammatory property and modulate the immune responses [13,14]. The biosynthesis of carotenoids is characteristic of the genus *Rhodotorula*. The chief carotenoids isolated from *Rhodotorula* species are reported to be torulene, torularhodin and carotene and small amount of carotene [15,16]. The use of bacterial pigments has doubled in the present decade because it offers various advantages over synthetic pigments [17]. Due to its fast growth, easy propagation and culturing, easy growth on cheap media sources and simple culture techniques, bacterial pigments are considered safe for human health. The increased usage of microbial pigments in various industries such as food, cosmetics, textile and pharmaceuticals has gained momentum [18].

Table 3. Antioxidant activity of *Staphylococcus sp*

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	0.517	0.551	0.628
2.	500 µg/ml	0.216	0.232	0.230
3.	250 µg/ml	0.244	0.247	0.245
4.	100 µg/ml	0.243	0.261	0.265
5.	50 µg/ml	0.269	0.266	0.270
6.	10 µg/ml	0.297	0.288	0.281
7.	Ascorbic acid	0.08	0.11	0.12

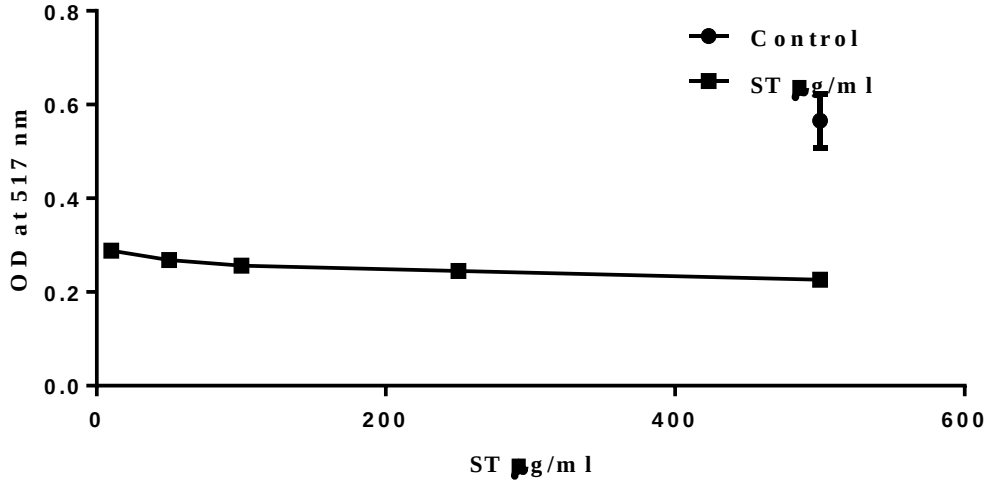

Fig. 5. Antioxidant activity of *Staphylococcus sp*

Table 4. Percentage of inhibition by pigments of *Staphylococcus sp*

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (%)
1.	Control	100
2.	500 µg/ml	59.99
3.	250 µg/ml	56.57
4.	100 µg/ml	54.62
5.	50 µg/ml	52.50
6.	10 µg/ml	48.90
7. a	Ascorbic acid	81.71

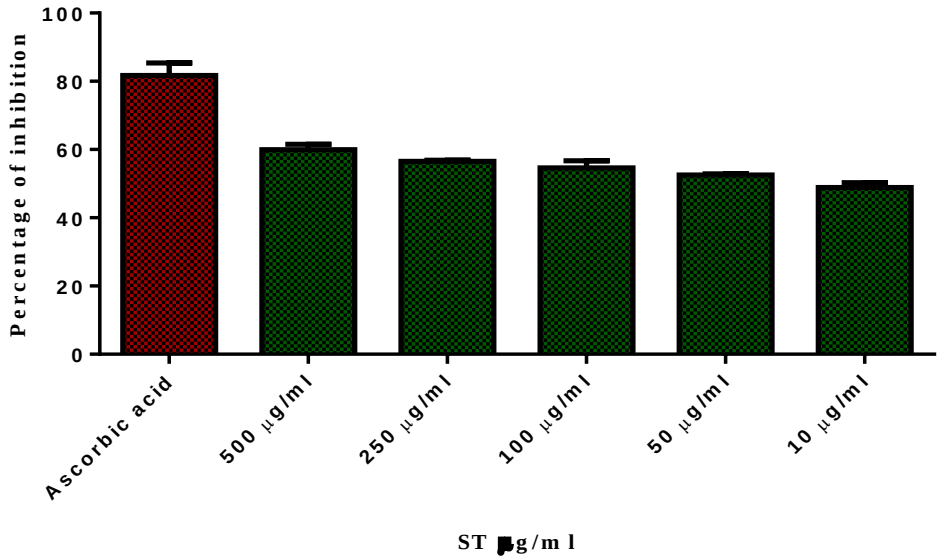

Fig. 6. Percentage of inhibition by pigments of *Staphylococcus sp*

Table 5. Antioxidant activity of pigments from *Rhodotorula sp*

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
8.	Control	0.404	0.384	0.394
9.	500 µg/ml	0.180	0.180	0.197
10.	250 µg/ml	0.188	0.205	0.212
11.	100 µg/ml	0.220	0.228	0.228
12.	50 µg/ml	0.251	0.250	0.271
13.	10 µg/ml	0.288	0.291	0.277
14.	Ascorbic acid	0.08	0.11	0.12

OD Value at 517 nm Control Mean OD value: 0.394

Table 6. Percentage of inhibition by *Rhodotorula sp*

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (%)
1	Control	100
2	500 µg/ml	52.87
3	250 µg/ml	48.81
4	100 µg/ml	42.80
5	50 µg/ml	34.68
6	10 µg/ml	27.57
7	Ascorbic acid	73.77

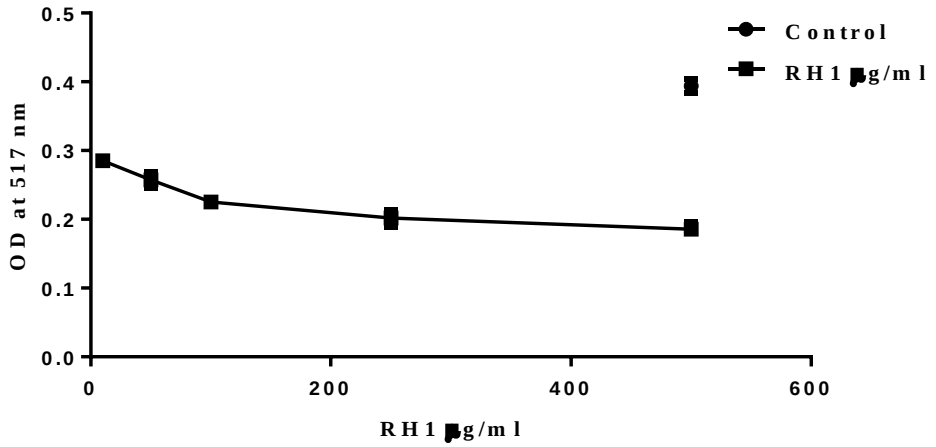

Fig. 7. Antioxidant activity of *Rhodotorula sp.*

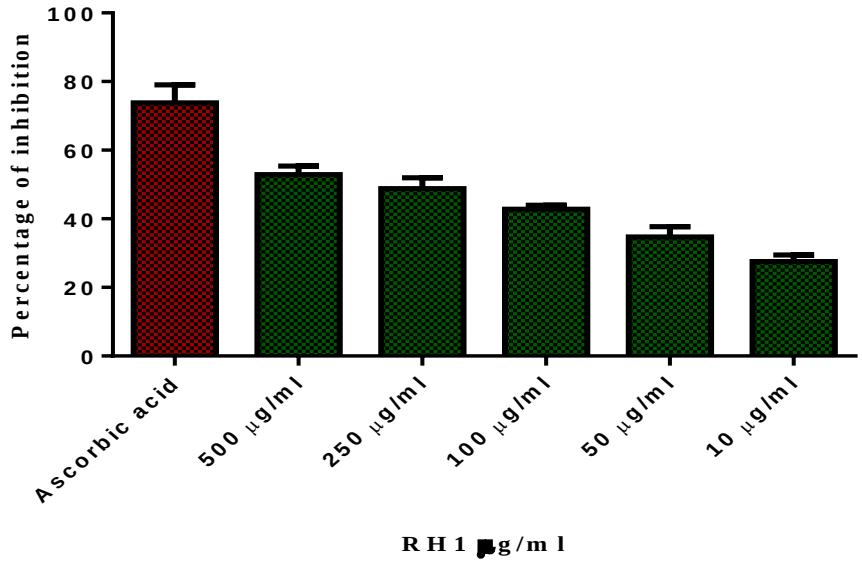

Fig. 8. Percentage of inhibition by pigments of *Rhodotorula sp.*

4. CONCLUSION

Our studies have established the antibacterial and antioxidant activity of pigments by *Rhodotorula sp* and *Staphylococcus sp*. Further characterisation of the pigments is required to provide a clear idea of the mode of action of the pigments. Future research on the biomedical potential in terms of clinical trials on animals and human beings should be carried out to uncover the real potential of therapeutic microbial derived pigments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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