

**Evaluation of Antioxidant Activity of *R. Slooffiae*, *R. Mucilaginosa* Extracts**Parichehr Hanachi<sup>1</sup>, Farzaneh Sadat Naghavi<sup>2</sup>

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**Type of article:** Original

**Abstract**

**Introduction:** Antioxidants are health beneficial compounds that can protect cells and macromolecules from the damage of reactive oxygen species (ROS). The aims of this study were to compare the total antioxidant and carotenoid production in *R. Slooffiae* and *R. Mucilaginosa*.

**Methods:** To isolate the carotenoid pigment, cells were suspended in acetone and broken using a homogenizer, followed by centrifugation, and supernatant was separated. For analytical method, pigments were measured spectrophotometrically at 450 nm. The B-carotene bleaching and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay were used to determine antioxidant properties of *R. Slooffiae* and *R. Mucilaginosa* by measuring the decrease in absorbance at 470 and 517 nm.

**Results:** The results showed that the content of total carotenoid in *R. Slooffiae* was higher than *R. Mucilaginosa* and it presented higher ability to show antioxidant activity. The mean total antioxidant activity of ascorbic acid was the highest ( $97.11 \pm 6.11\%$ ), followed by BHT ( $64.71 \pm 5.41\%$ ), *R. slooffiae* extract ( $57.91 \pm 7.34\%$ ) and *R. Mucilaginosa* ( $39.32 \pm 5.85\%$ ). The EC<sub>50</sub> of ascorbic acid was the strongest ( $0.252 \pm 0.000$  mg/ml), followed by BHT ( $0.612 \pm 0.009$  mg/ml) and *R. Slooffiae* ( $0.658 \pm 0.033$  mg/ml). There was significant difference observed between the EC<sub>50</sub> of *R. Slooffiae* and BHT.

**Conclusion:** It was found that both strains have ability to produce carotenoid and show antioxidant ability; however, *R. Slooffiae* had more potential in producing carotenoid and showing antioxidant ability than *R. Mucilaginosa*. Further study is required, in order to utilize this strain in the food, pharmaceuticals and cosmetics industries.

**Keywords:** Antioxidant, Carotenoid, Rhodotorula, Radical scavenging activity

**1. Introduction**

The energetic benefit of aerobic metabolism is associated with the generation of reactive oxygen species capable of damaging biologically relevant molecules such as DNA, protein and lipid, which has been termed "Oxidative Stress" and numerous varieties organisms may encounter it as a normal attribute of aerobic life. (1, 2). Carotenoid pigments, including carotenes and xanthophylls, are very widely distributed in nature. They contain an extended system of conjugated double bonds, which is responsible for their antioxidant properties and they were classified as a Nonenzymatic antioxidant (3). This latter property is closely related to their ability to decrease risks of a variety of degenerative diseases such as cancer, cardiovascular disease, macular degeneration and cataract (4). In ageing yeast cells, carotenoids preserve viability by defending cells against oxygen radicals, and perhaps, compensating the lack of specific antioxidant enzymes (5, 6). The antioxidant action of carotenoids against reactive oxygen species and particularly against  $1O_2$  is well documented (7, 8). Access to natural and economical resources, to produce supplements and antioxidant drugs, can be a great help in the prevention and treatment of the results of oxidants in the body. The aims of this study were to determine and compare the total antioxidant and carotenoid production in

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*R. Slooffiae* and *R. Mucilaginoso*, which might be considered as a possible source for antioxidative substances for commercial or pharmaceutical purposes.

## 2. Material and Methods

### 2.1. Cultures and cultivation

The red yeast strains were used from the private culture collection at the National Laboratory of Industrial Microbiology, Alzahra University, Tehran (Iran). Samples were maintained in Petri dishes containing Yeast Extract-Peptone-Glucose agar medium (YPG agar) [ $\text{g l}^{-1}$ ]: yeast extract 5, agar 17, peptone 10, dextrose 20, and pH = 6.

### 2.2. Carotenoids analysis

Samples were prepared by cultivation of the yeast strain on YPG agar at 30 °C for 48 h. transferring one full loop into YPG broth incubating on a rotary shaker at 150 rpm and 30 °C overnight (9). The methods of Davis and Naghavi, with modification, were used for the extraction of carotenoid pigments. The total carotenoid was determined spectrophotometrically using the extinction co-efficiency of  $A^{1\%}_{1\text{cm}}=2500$ , as proposed by Davis (8, 10).

### 2.3. DPPH radical scavenging method

Effects of *R. slooffiae*, *R.mucilaginoso* extract on DPPH radical activity were measured according to the method described by Yen and Hesieh, 1998 (11).

### 2.4. $\beta$ -carotene bleaching

Antioxidant activity of BFE was measured according to the  $\beta$ -carotene bleaching method described by Gazzani et al., 1998 (12). The total antioxidant activity, which reflected the ability of the Yeast extracts to inhibit the bleaching of  $\beta$ -carotene, was measured and compared with standards.

### 2.5. Statistical analysis

All determining values were subjected to analysis of mean, using one-way variance analysis (ANOVA). The Statistical Package for Social Science for windows version 11.5 was used to analyze the data. Values  $p<0.001$  were considered to be significant.

## 3. Results

### 3.1. Carotenoids analysis

Table1 shows the amount of biomass and total carotenoids of both strains. *R. Slooffiae* and *R. Mucilaginoso*. Each strain has the capacity to produce carotenoids. *R. Slooffiae* significantly ( $p<0.001$ ) presented a higher value of carotenoid production and concentration than *R. Mucilagino*a after 72 hours incubation.

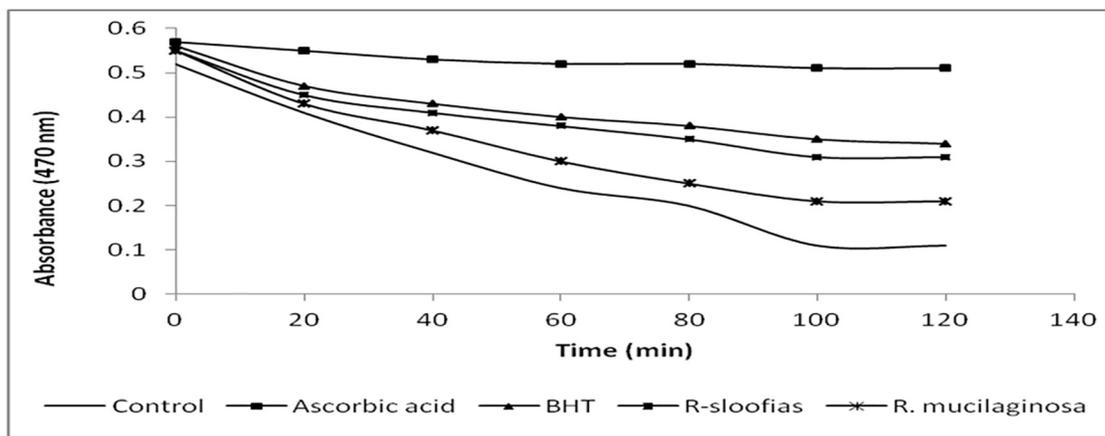
**Table 1.** Amount of biomass and total carotenoid of two strains were grown in 100 ml of MMS broth in a 500-ml Erlenmeyer flask incubated on a rotator shaker at 150 rpm and 30 °C for 72 hours.

Strains	Dried cell mass ( $\text{g l}^{-1}$ )	Carotenoid concentration ( $\text{mg g}^{-1}$ )	Total antioxidant
<i>R. Slooffiae</i>	1.660 $\pm$ 0.078	1.216 $\pm$ 0.054	57.91* $\pm$ 7.34
<i>R. Mucilaginoso</i>	0.968 $\pm$ 0.094	0.604 $\pm$ 0.082	39.32* $\pm$ 5.85

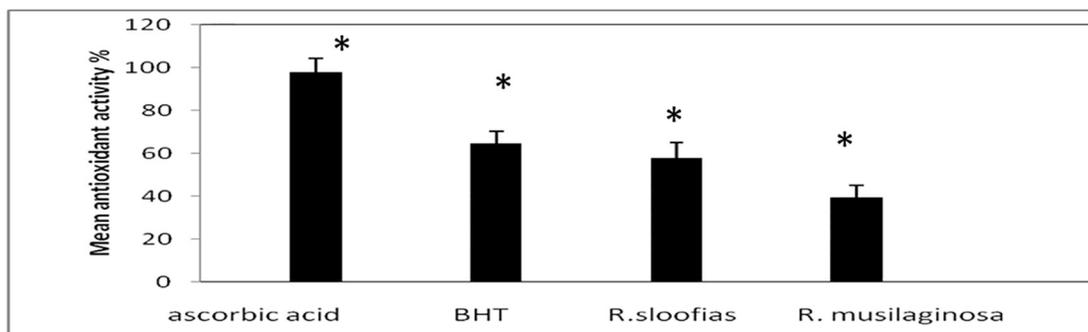
\*Significant level was set at  $P<0.05$ . Data are expressed as mean  $\pm$  SD.

### 3.2. $\beta$ -carotene bleaching

The total antioxidant activity, which reflected the ability of the extract to inhibit the bleaching of  $\beta$ -carotene, was measured and compared with standards. The  $\beta$ -carotene bleaching rates are shown in Figure 1. It showed a decrease in absorbance of  $\beta$ -carotene in the presence of different samples due to the oxidation of  $\beta$ -carotene. As shown in Figure 2, the mean total antioxidant activity of ascorbic acid was the highest (97.11  $\pm$  6.11%), followed by BHT (64.71  $\pm$  5.41%), *R. slooffiea* extract (57.91  $\pm$  7.34%) and *R. mucilaginoso* (39.32  $\pm$  5.85%).



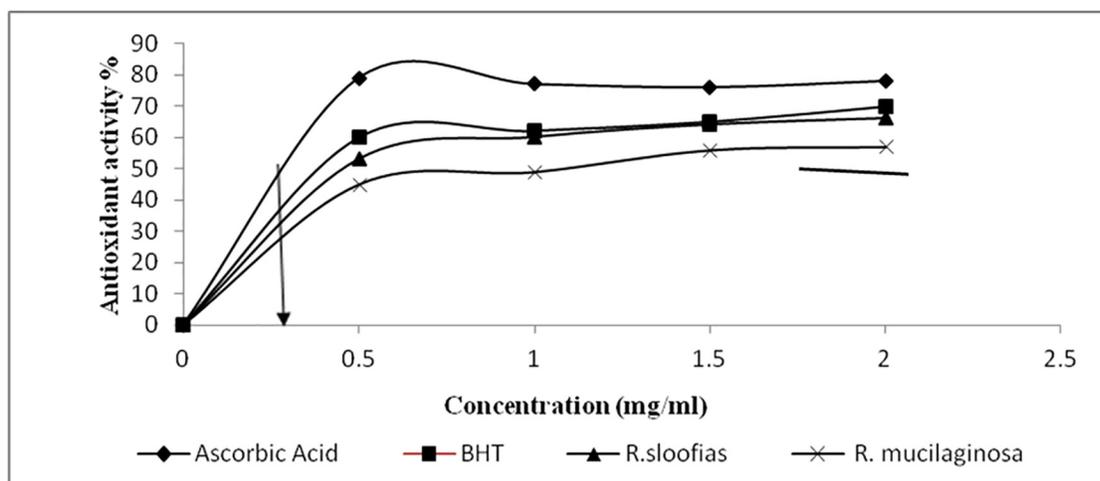
**Figure 1.**  $\beta$ -carotene bleaching rates of, *R. slooffiae*, *R. mucilaginosa*, extract compared with ascorbic acid and butylated hydroxyl toluene (BHT) at 1 mg/ml  $\beta$ -carotene chloroform.



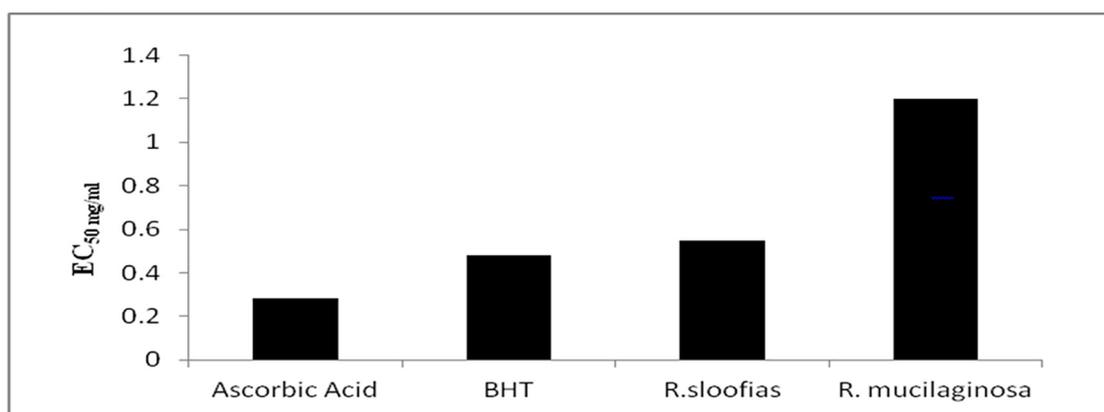
**Figure 2.** Mean total antioxidant activity of *R. slooffiae*, *R. mucilaginosa*, extract, ascorbic acid and butylated hydroxyl toluene (BHT) measured by  $\beta$ -carotene bleaching assay. The (\*) indicate values significantly different with  $p > 0.05$ . Results are means of three determinations.

### 3.3. DPPH free radical scavenging

The free radical scavenging activity of studied sample extract, and standards at different concentrations are presented in Figure 3. Figure 4 shows the comparison of the mean concentration of 50% free radical scavenging activity ( $EC_{50}$ ) of sample and standards against 250  $\mu$ M DPPH radical. The  $EC_{50}$  value was defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50 % in comparison to control. The  $EC_{50}$  of ascorbic acid was the strongest ( $0.298 \pm 0.000$  mg/ml), followed by BHT ( $0.452 \pm 0.009$  mg/ml), *R. Slooffiae* ( $0.658 \pm 0.033$  mg/ml) and *R. Mucilaginosa* ( $0.789 \pm 0.0125$ ). There was no significant difference observed between the  $EC_{50}$  of *R. Slooffiae* and BHT. As shown in Figure 2, the mean total antioxidant activity of ascorbic acid was the highest ( $97.11 \pm 6.11\%$ ), followed by BHT ( $64.71 \pm 5.41\%$ ), *R. Slooffiae* extract ( $57.91 \pm 7.34\%$ ) and *R. Mucilaginosa* ( $39.32 \pm 5.85\%$ ). There is no significant difference observed between *R. Slooffiae* extract and BHT. However there was significant ( $p < 0.05$ ) relation between total antioxidant percentage and ascorbic acid compared *R. Slooffiae*, *R. Mucilaginosa* and BHT.



**Figure 3.** Scavenging activity of ascorbic acid, butylated hydroxyl toluene (BHT) and *R. slooffiae* and *R. mucilaginosa* extract on DPPH radical.



**Figure 4.** Free radical scavenging activities (EC<sub>50</sub>) of *R. slooffiae* and *R. mucilaginosa* extract, ascorbic acid and butylated hydroxyl toluene (BHT). The DPPH radical concentration was 0.250 mM in all reaction mixtures. The same letter (b) indicates values not significantly different from each other with  $p > 0.05$

#### 4. Discussion

This study revealed that both strains, *R. Slooffiae* and *R. Mucilaginosa*, are able to produce carotenoid pigments and these pigments have a significant antioxidant effect. The results for comparison of the amount of total carotenoid in two selected strains at 72 hours showed that *R. Slooffiae* and *R. Mucilaginosa* differ in their carotenoid content and *R. Slooffiae* had a greater ability to produce carotenoids, which are confirmed by another research (13). In the present study, the antioxidant effect of extracts was determined using *B-carotene bleaching* and DPPH. Studies show that carotenoids have an antioxidant property and a defense function in yeasts, due to this property (8, 14-16). For instance, in some yeast such as *Rhodotorula Mucilaginosa* it has been indicated, carotenoids protected the organism against experimentally induced oxidative damage (17). The production of carotenoids by genus *Rhodotorula* is affected by species, medium constituents and environmental conditions (18). The properties of antioxidants formed by yeasts can be substantially influenced by the environment surrounding the cells. Results of previous experiments suggested the increase in the amount of carotenoid pigments result in an exposure to oxidative stress (19). In this study, due to the equivalent environmental situation for both examined strains, we might assume that the excellent antioxidant ability of *R. Slooffiae* was associated with high intrinsic ability to produce carotenoid. However, in another research we found the production of carotenoid in these strains highly dependable on time of incubation; in other words, incubating *R. mucilaginosa* at its optimum incubation time (48 h) can set it as a potential radicals scavenger (13). Even though, in a Chen et al (20) assay, the ability of DPPH scavenging capacity of 12 yeast strains included 2 strains of *R. Mucilaginosa*.

## 5. Conclusions

There has been an exponential rate of interest in the role of oxygen-free radicals, more generally known as “reactive oxygen species” (ROS) and of “reactive nitrogen species” (RNS) in experimental and clinical medicine. ROS/RNS are known to play a dual role in biological systems, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS at low concentrations involve roles in defense against infectious agents and in the function of a number of cellular signaling systems. Results showed all strains other than one type of *R. mucilaginosa* exhibited antioxidant capacity. However, *Rhodotorula spp.* showed less antioxidant property than others.

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## Conflict of Interest:

There is no conflict of interest to be declared.

## Authors' contributions:

Both authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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