NEC
NUTRIMENTUM ET CURAE

Nutr Cur 2024; 03: e155
DOI: 10.57625/nec.2024.73

Fermentative production, comparative characterization with carrots, and functional analysis of β-carotene produced from *Rhodotorulla toruloids*

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ABSTRACT

INTRODUCTION. Beta-carotene, a widely demanded red-orange pigment, has multiple applications in the food, cosmetic, and textile industries. Synthetic pigments are associated with harmful side effects necessitates the exploration of natural alternatives. *Rhodotorula toruloides*, a yeast strain, offers a promising natural source of β-carotene, potentially overcoming the limitations posed by traditional plant-based sources like carrots.

MATERIALS AND METHODS. This study β-carotene was extracted from *R. toruloides* merged fermentation with YPD, optimized for maximum pigment production. Characterization was performed using spectrophotometry, Thin Layer Chromatography (TLC), and High-Performance Liquid Chromatography. The antimicrobial and antioxidant activities of the produced β-carotene were analyzed, and its suitability as a dye was tested.

RESULTS. The yield of β-carotene from *R. toruloides* was 0.36 g/L with significant antimicrobial activity against *Salmonella* (25.3 ± 0.3 mm inhibition zone), outperforming carrot-derived β-carotene. Additionally, the DPPH assay revealed strong antioxidant activity. The β-carotene from *R. toruloides* also successfully dyed cotton fabric, demonstrating its potential as an organic dye.

CONCLUSIONS. The study concludes that β-carotene from *R. toruloides* exhibits superior antimicrobial and antioxidant properties compared to carrot-derived β-carotene. With its ease of cultivation and cost-effectiveness, *R. toruloides* presents a viable, natural, and efficient alternative source for β-carotene production, suitable for various industrial applications.

KEYWORDS

BETA-CAROTENE
RHODOTORULA TORULOIDES
ANTIMICROBIAL PROPERTIES
ANTIOXIDANT PROPERTIES

INTRODUCTION

Carotenoids are composed of isoprene units forming a 40-carbon polyene skeleton with three to fifteen double bonds, which are responsible for their significant coloration. They are abundantly present in *Daucus carota* (carrots) and some other plants1. Among carotenoids, β-carotene, a strong red-orange pigment, has gained immense importance due to its rising demand in pharmaceutical, medical, cosmetics, food, and textile industries[2].

In 2018, the global production value of β-carotene[3] was approximately $309 million, with an annual growth rate of 3.6%. This demand is expected to increase by 2.9% annually. By 2023, the global market for β-carotene[4,5]

was estimated at 303.8 million USD and is projected to reach 372 million USD by the end of 2030. However, synthetic pigments have several side effects, including causing allergies, toxicity, and even cancer. This has led the scientific community to explore natural sources of pigments[6,7].

Several plants and fruits, such as carrots, tomatoes, oranges, grapefruits, and olives, contain high concentrations of β-carotene. However, being seasonal and requiring long growth periods, they are not suitable for commercial-scale pigment production. Microorganisms such as fungi and algae can serve as alternative sources for β-carotene production8. Several yeasts, including *Phaffia*, *Rhodotorula*, and *Sporobolomyces*, and filamentous fungi like *Blakeslea trispora*, are reported as efficient β-carotene producers in the presence of significant inducers such as dextrose[9-11].

Yeasts produce β-carotene as a secondary metabolite during their stationary phase, and the production can be scaled up via fermentation by providing optimized conditions such as suitable temperature, pH, and sugar source[12,13]. Biotechnological production of natural colors using low-cost substrates like agro-industrial residues is an economical approach for natural pigment production[14]. In this regard, the present study aimed to isolate β-carotene-producing fungi followed by its fermentation to produce β-carotene. The functional analysis of β-carotene was also conducted to determine its antimicrobial and antioxidant potential, with its ability to dye cotton, compared to β-carotene extracted from carrots.

MATERIALS AND METHODS

Isolation and Characterization

Oranges (*Citrus sinensis*) were collected for fungal cultivation and placed for 15 days under dark conditions to rot. The rotten peels were scraped, crushed, and shaken in sterile saline water. The suspension of this prepared sample was inoculated in sterilized Sabouraud Dextrose Agar medium (SDA, pH 6.5) and incubated for 5 days at 30°C. After incubation, pink-colored colonies were selected for purification culturing on Yeast Peptone Dextrose Agar (YPDA) medium to obtain the pure culture. The isolated pink-pigmented pure culture was characterized at morphological, microscopic, and biochemical levels by colony morphology examination, lactophenol cotton blue staining[15], and carbohydrate utilization test[16], respectively. For molecular characterization, the strain was further sent to the School of Biological Sciences (SBS), Punjab University Lahore, for 18S rRNA sequencing.

Optimization Conditions for Fermentation

Different fermentation parameters, such as culture media, carbohydrate source, temperature, and pH, were optimized for maximum pigment production.

Optimization of Cultivation Media

Three different culture media were used for the cultivation of the isolated strain:

- **Basal media:** 2% glucose, 0.5% yeast extract, 0.1% KH_2PO_4, 0.05% $MgSO_4$
- **MS3:** 3% glucose, 15% yeast extract, 0.1% KH_2PO_4, 0.04% $MgSO_4$, 0.5% NH_4NO_3, 0.4% NaCl, 0.04% L-alanine;
- **Yeast Malt extract medium:** 1% glucose, 0.3% yeast extract, 0.5% peptone, 1.5% malt extract; pH 6.2.

The cell density in each incubated medium was estimated by measuring optical density at 600 nm every 24 hours[17].

Optimization of Sugar Source for Pigment Production

Three different propagation media with the composition of 1% yeast extract, 2% peptone, 2% sugar, and agar were used to analyze maximum pigment production. The sugar constituent varied in each medium:

- **Yeast Peptone Dextrose (YPD) agar:** 2% dextrose; pH 6.8.
- **Yeast Peptone Glucose (YPG) agar:** 2% glucose; pH 6.5.
- **Yeast Peptone Sucrose (YPS) agar:** 2% sucrose; pH 6.0.

Each medium was inoculated with fresh inoculum and grown in optimized cultivation media for five days. The optical density of each batch[18] was observed after every 24 hours of incubation at 450-500 nm.

Optimization of Various Production Parameters

Optimization of different parameters is essential to assess the best conditions for pigment production. Response surface methodology (RSM) was employed with five levels of central composite rotatable design (CCRD). The combined effect of three independent variables was examined:

- **pH range** of 6-10 (X_1).
- **Sugar concentration** of 2-6% dextrose (X_2).
- **Temperature range** of 20-30°C (X_3).

These were checked on the response variable, highest pigment yield (Y_1). A significant relationship among the variables was predicted via a second-order polynomial quadratic model.

Fermentative Production of Pigment
After optimization of all parameters, submerged fermentation was carried out to produce the pigment. About 3% of inoculum, cultured in basal media with predetermined cell density and cell count (5.6×10^8 CFU/mL), was added to 100 mL of YPD media and incubated at 25°C for 120 h. After fermentation, the content was centrifuged at 4000 rpm for 15 min. The supernatant was discarded, and the pellet was washed with distilled water, dried, and stored for further analysis.

Downstream Processing
The pigment produced during the fermentation process was further extracted, purified, and characterized.

Pigment Extraction and Partial Purification
The pigment present in dried yeast cells was extracted by mixing it with DMSO and then subjected to 10 minutes of discontinuous sonication at 20 kHz. Ten-second pauses were maintained to ensure complete cell disruption and maximize pigment collection in the solvent (DMSO). After sonication, the processed DMSO was mixed with 99.9% pure chloroform for partial purification of the pigment[19]. The chloroform layer separated and subjected to evaporation. For com purification, the dried product was mixed with 9 methanol. The pigment-containing layer was separated, dried, and weighed to obtain the pigment yield. Alongside pigment production from th urce, β-carotene was also extracted from ca , a r -carotene source. The idea was to compa e eff yeast-extracted β-carotene and ca p-ca tene. The crushed carrots were dipped CaCO₃ solution, which was further cted to ex- traction. The filtrate was ev ted and dried t obtain powdered β-carotene[20].

Pigment Characteriza n
The characterizati of the extracted ment was carried out by spe photometry and chromatographic techniques. U sibl pectroscopic analysis was done in the l ran 450- nm to determine the maximum a peak[21] n-layer chromatography (TLC) anal cted using Silica Gel 60 F_{254} gl lates (2 , and a mobile phase solven e and dis ed water in a 3:1 ratio was u o ch te the pigment based on Rf value cal- ed u ula[22]:

\[\te \frac{\text{Distance traveled by the compound}}{ Distance traveled by the solvent}} \]

Fourier-transform infrared (FTIR) spe nal- ysis was conducted using an FTIR s opi r (Thermo Nicolet Model-iS50) wit ectral ran 15-35,000 cm^{-1} at Applied Chemis rch Ce PCSIR, Lahore[23]. For high-performan o- matography (HPLC), the s rd and t cted pigment were prepared u acet . A C18 olumn with a mobile phase o eton :2-p panol:ethyl acetate (40:40:20) at a 1 mL was used for the detection of the ex ign .

Biological Acti Analysis of ent
Microbial pi are in high demand in medicinal, food, and t s due to their eco-friendly nature and of cu Besides coloration, they possess significant biol activities. Therefore, the antim al and antioxidant activities of the extracted pi ere assessed, along with its dyeing ability.

icrobial vity
T imicro potential of the extracted pigment aga *coccus sp.*, *Salmonella sp.*, and *E. coli* was teste ing the agar well diffusion method[25].

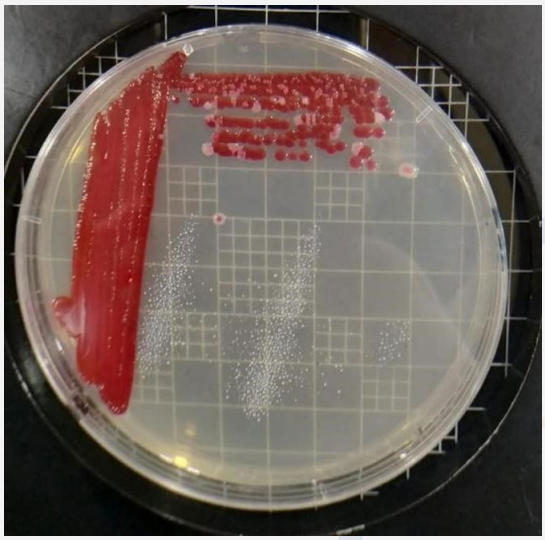
nt Activity
ant activity analysis was performed using the 2-diphenyl-1-picrylhydrazyl (DPPH) method. DPPH was first mixed with methanol and homogenized under ark conditions to prevent radical oxidation. Yeast-derived β-carotene (50 µL) and carrot-extracted β-carotene (100 µL) were added to separate mixtures of DPPH and methanol, followed by incubation for 2-3 min until color change. Samples were then subjected to spectrophotometry; absorbance was recorded at 520 nm, and data were plotted with concentration on the x-axis and percentage antioxidant activity on the y-axis[25].

Dyeing Ability of Pigment
To evaluate the application of the extracted pigment in dyeing, cotton fabric was dyed in a defined ratio of pigment, organic solvent, and water. The extracted pigment was dissolved in dilute acetone, and the pH of the dye bath was adjusted to 6.5 and the temperature to 40°C for a few hours. The fabric was then washed to remove any excess dye[26].

RESULTS

Isolation and characterization of fungal strain
In the present study, pink-colored microbial colonies, suspected to be the producers of β-carotene, were isolated from rotten orange peels on PDA. The morpho-

logical characteristics, i.e., pink, mucoid, and round colonies (Figure 1a), as well as the microscopic characteristics of the isolated strain, indicated it as yeast. Further screening was conducted based on biochemical tests. As carbohydrate is the main inducer of β-carotene production, the ability of isolated yeast strains to ferment different sugars (dextrose, fructose, sucrose, and glucose) was estimated. The results of the carbohydrate assimilation test showed that the yeast [illegible]ated glucose and dextrose with gas produ[illegible], ind[illegible]y a color change from red to yellow[illegible] bubble f[illegible] tion in Durham tubes. Finally, 18[illegible]sequen[illegible] was conducted for phylogenetic analys[illegible]ed strain. The results of 18S rR[illegible] sequencing [illegible]iso-lated strain showed 100% [illegible]lari[illegible]with Rho[illegible]torula toruloids, as indicated i[illegible]gure [illegible].



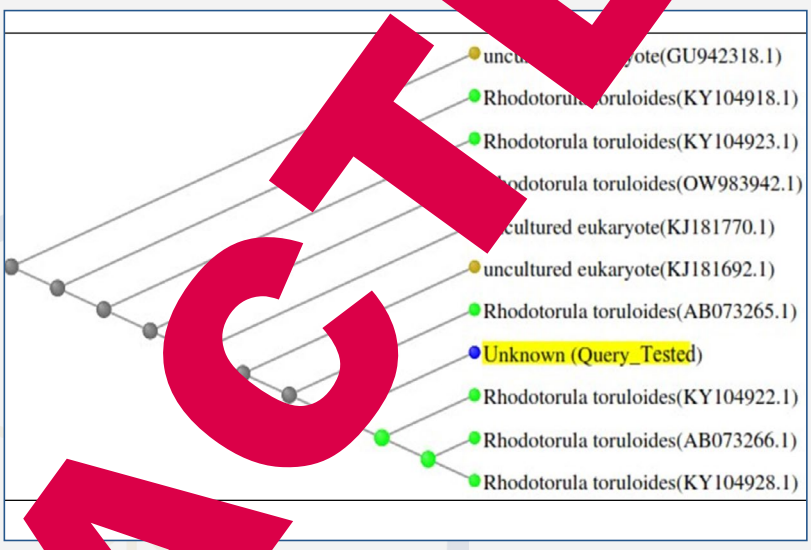


Figure 1. Isolation and characterization of yeast. a) Isolate[illegible]ored round, mucoid entire and smooth microbial colonies. b) Phylogenetic tree showing 100% similarity of an isolated y[illegible]rain with *Rhodotorula torulo*.

Optimization conditions for ferme[illegible]ion

To maximize the yield of pigment[illegible] parameters such as culture media (Bas[illegible]and YM media), sugar source (Dextros[illegible]ucose, [illegible]se), and physical parameters ([illegible]d temperatu[illegible]were optimized. Among the t[illegible]ltivation media, basal media facilitated maxi[illegible]rowth (1.83±0.02 OD at 600 nm) withi[illegible]0 hou[illegible]ubation as indicated in (Figure 2a). Maximum p[illegible]t production was induced by d[illegible]ose as maximum OD was recorded in YPD me[illegible]fter [illegible] hours of incubation. Figure 2b shows the[illegible]ct of [illegible]erent [illegible]ugar-containing media on pigmen[illegible], me[illegible]d via OD at 460 and 490 nm respec[illegible]. Respon[illegible] surface [illegible]ogy (RSM), employing a cent[illegible]site desig[illegible]CD), was utilized to optimize th[illegible]ndi[illegible] for maximum β-carotene yield. The optic[illegible]cond[illegible]imum yield of β-carotene produc[illegible]ntione[illegible] Table 1 and indicated in Figure 3. The[illegible]evealed that pH had no significant effect on pigmen[illegible]whereas temperature exerted a notable effect, with[illegible]ptimal production occurring at 25°C and declining thereafter. Additionally, sugar concentration significantly impacted pigment yield, with maximum production observed at 4% dextrose.

In the ANOVA quadratic model (Table 2), the F-value of 1.42 suggests that the model lacks significance compared to noise, with a 29.37% probability of this F-value arising due to noise. Factor coding was performed, and model terms were deemed significant if the p-value was below 0.0500. However, no significant model terms were observed in this case, indicating the need for model reduction. The standard plot of the pH effect on pigment extraction indicates no significant effect on pigment yield, while the curve in the standard plot of temperature effect indicates its significant effect on pigment yield. The graph shows minimum β-carotene yield at a lower temperature, maximum at 25oC, and again less at a higher temperature. The effect of sugar concentration on the yield of pigment showed a straight-line standard curve, indicating maximum pigment production at 4%. The final equation provided by Design Expert-V 11 allows the prediction of β-carotene yield based on specified levels of pH, temperature, and sugar concentration.

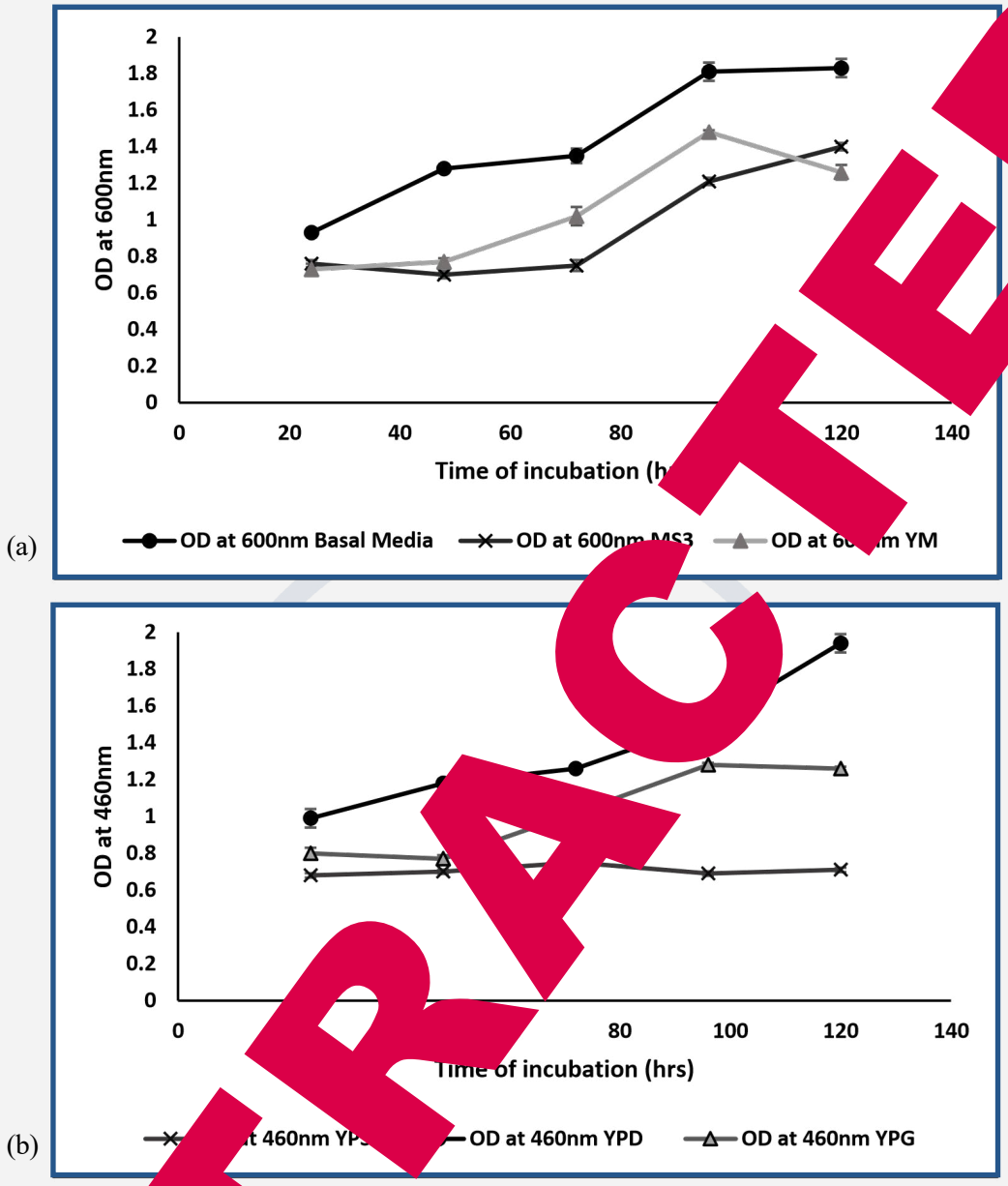


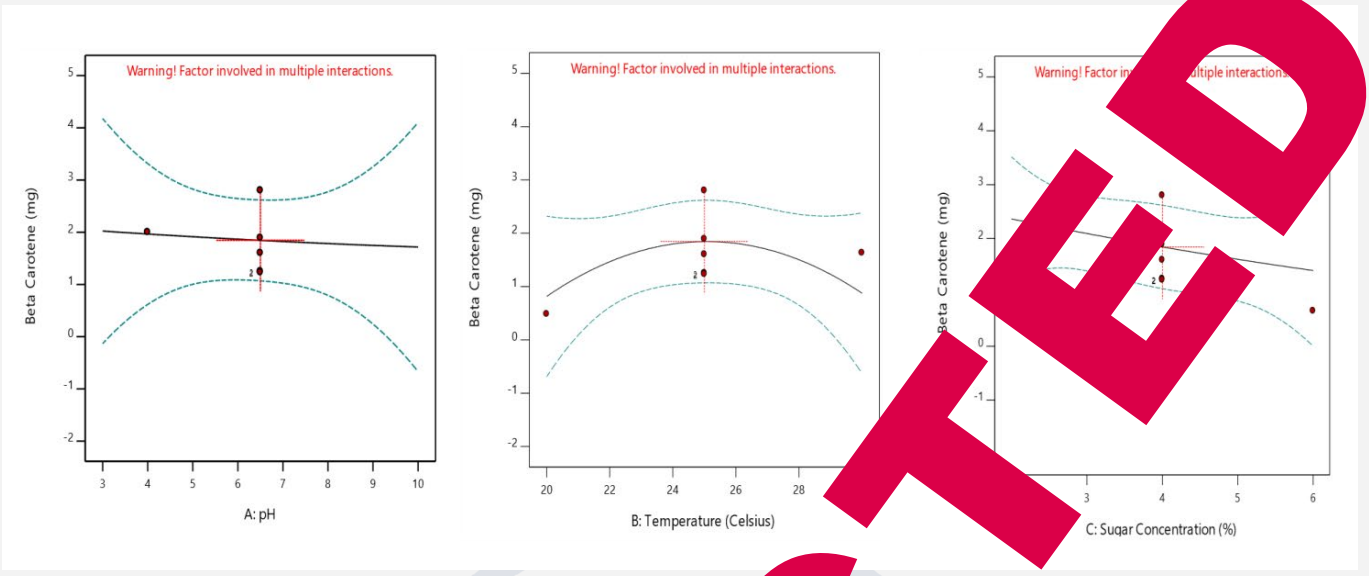
Figure 2. Optimization c ulture m ultivation media optimization on the basis of *R. toruloids* cell density in terms of OD measured at 600 m. Basal media w own to facilitate higher cultivation of *R. toruloids*. b) Optimization of media for β-carotene produc ndicated in a graph showing peaks of maximum pigment production in terms of OD measured at 460 nm.YPD media show facilitate maximum β-carotene production.

Table 1. Optimized experimental design for β-carotene production.

Std	Run	Factor 1: A-pH	Factor 2: B-Temperature (Celsius)	Factor 3: C-Sugar Concentration (%)	Response 1 [illegible]rotene (n
3	1	3	30	2	
6	2	10	20	6	0.
7	3	3	30		1.5
19	4	6.5	25		.23
1	5	3	20	2	2.39
9	6	6	25	4	2
17	7	6.5	25	4	1.25
18	8	8	25		3.6
15	9	6.5	25		1.6
14	10	10		6	0.655
5	11	3		6	0.32
4	12	10	25	4	0.76
13	13	6.5		2	1.35
11	14	6.5		4	1.63
8	15	15	30	6	0.46
10	16	8		2	3.31
2	17	10		4	0.945
16	18	8	25	4	1.89
12	19		20	4	0.48
20	20		25	4	2.8

Table 2. Response of β-carotene based [illegible] quadratic model.

Source	Sum of Squa[illegible]	B[illegible]	Mean Square	F-value	*p*-value	Significance
Model	10.5[illegible]	*9*	1.18	1.42	0.2937	Not significant
A – pH	0.[illegible]	1	0.1972	0.2387	0.6357	Not significant
B – Temperature	0.[illegible]93		0.0093	0.0113	0.9176	Not significant
C – Sugar Concentration	2.[illegible]54	1	2.54	3.08	0.1098	Not significant
AB	[illegible]188	1	0.1188	0.1438	0.7124	Not significant
AC	0.155[illegible]	1	0.1552	0.1879	0.6739	Not significant
[illegible]		1	1.72	2.08	0.1801	Not significant
[illegible]	0.[illegible]08	1	0.0008	0.0009	0.9764	Not significant
[illegible]	[illegible].99	1	1.99	2.41	0.1513	Not significant
[illegible]	0.0041	1	0.0041	0.0050	0.9452	Not significant
[illegible]	8.26	10	0.8262			
Lack [illegible]	6.55	5	1.31	3.84	0.0830	Not significant
Pure Error	1.71	5	0.3414			
Core Total	18.86	19				



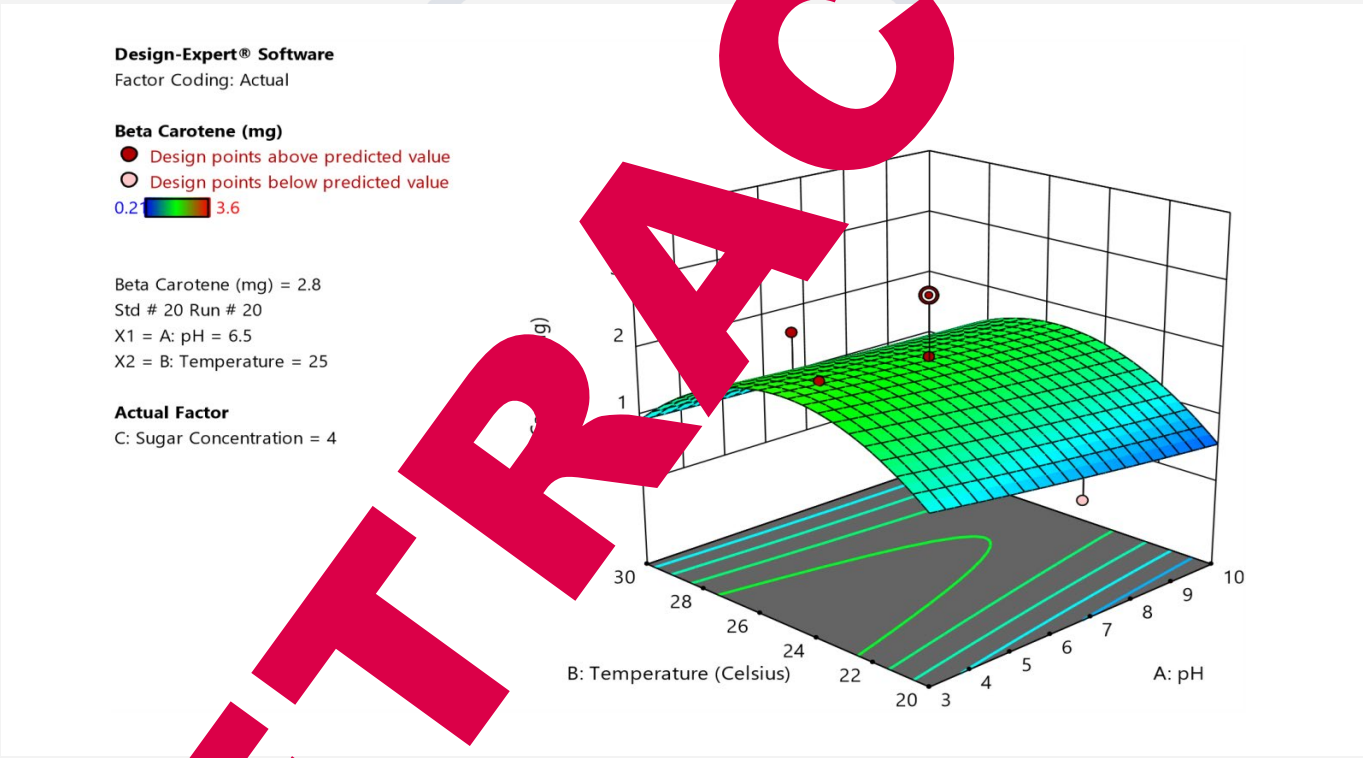


Figure 3. Re su meth ogy plots achieved through design experts where RSM plots showing the optimization of three variables Temp re °C (c) Sugar Concentration for maximum production of β-carotene. The plot indicated 2.8 mg β-carotene pro sugar concentration as 4%, pH as 6.5, and temperature at 25°C.

Beta-Carotene = -16.52492-0.166324 pH+1.77265 Temperature

-1.35210 Sugar Concentration+0.006964 pH * Temperature

-0.019668 pH * Sugar Concentration

+0.046313 Temperature * Sugar Concentration

+0.002095 pH²-0.039941 Temperature²

+0.010361 Sugar Concentration²

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor, and the intercept is not at the center of the design space.

Pigment extraction, purification, and characterization

After the optimization of parameters, pigment production was conducted via submerged fermentation taking the inoculum of cell density of 0.57 OD at 600 nm cell count of 5.6×108 CFU/ml in YPD media inc ing for 120 hours at 25°C in the static incubator. A fermentation, yeast biomass (0.6 g/L) was separate via centrifugation, and the pellet was subjected to solvent-based extraction using chloroform hanol to get pigment as shown in Figure 4a. eo -car-otene was also extracted from crush arrot cated in Figure 4b.

The maximum yield of β-carotene was 36 g/L and 1.58 g/kg from yeast and ca resp A higher yield of β-carotene from s may ind its worth, but *R. toruloids*, being ilable cost-effective production can rove a b e source as compared to carro Characterization of the e cted igment w con-ducted using spectrop met LC, FTIR, and HPLC respectively. T notor analysis revealed that standard β- abs d maximum wavelength at 46 and 49 optical density (OD) of *R. to s* extracted p otene was mea-sured as 0.56 60 while that of carrot extracted β-carotene 0.59 at 460 nm and 490 nm respectiv s indi igure 5.

Thin layer chromatogra TLC) was performed for the d on of extracted pigment based on retarda-ti (Rf) in a solvent system of diluted acetone one and in 3:1). The Rf value of pigment cted from t and carrots was calculated as 0.91 a respec y (Figure 6) as compared to that of sta ene (Rf =0.92)[27].

For further confirmation and characterization of ex-cted pigment, its FT IR analysis and HPLC were d.

IR analysis indicates the presence of specific nctional groups in the tested compound. For β-car-otene, the FTIR spectra typically reside in the range f 3500 cm^{-1} to 700 cm^{-1}. While comparing the IR spectra of yeast and carrot extracted β-carotene with the standard β-carotene, three types of key peaks were identified. Firstly, a peak between 3000 cm^{-1} - 2500

Figu ed and dried pigment. (a) β-carotene extracted from *R. toruloids*. (b) β-carotene *extracted from carrots*.

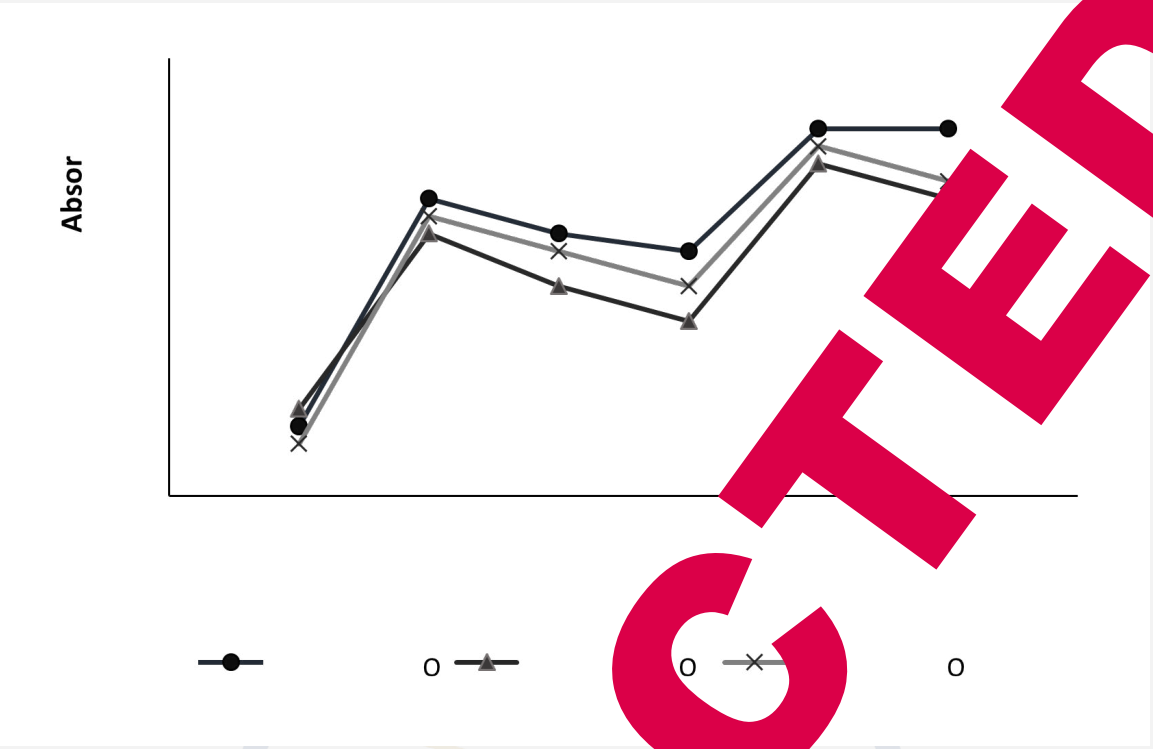


Figure 5. Absorbance of pigments. The graph indicates [illegible] photometric analysis of carrot extracted β-carotene (pigment 1), *R. toruloids* carrot extracted β-carotene (p[illegible] with standard β-carotene.

(a) (b) (c)

Figure 6. TLC analysis of (a) β-carotene standard, (b) pigment extracted from yeast, and (c) pigment extracted from carrots respectively under UV at 354 nm.

cm^{-1} indicated the presence of C-H stretches in all three spectra as indicated in Figure 7. The second significant peak resided in the range of 1600 cm^{-1} to 1700 cm^{-1}, representing C=C stretching. However, peaks in this region were observed at different wavenumbers for each sample, i.e., 1629 cm^{-1} for the microbial pigment, 1713 cm-1 for carrots, and 1637 cm^{-1} for standard β-carotene. This suggests slight variations in the chemical structure or molecular environment of the C=C bonds in each sample. Furthermore, the di-substitution in the C=C. group was detected at 935 cm^{-1} for microbial pigment, 964 cm-1 for carrots, and 1003 cm^{-1} for standard β-carotene. The overall IR spectra confirmed the successful extraction of β-carotene[28].

Finally, the samples were further ch… via HPLC. The β-carotene from yeast a… rrots … against HPLC grade standard β-ca… The Pea… the HPLC spectrum were recorde… RT of 6… mins for standard β-carotene while … 29 mins for *R. toruloids* extra… β-carotene… rrot extracted β-carotene, resp…ely … hown in F…gure 8. The noise that appeare… the …tra o… carrots and yeast is caused by t… of sl… impurities or other compounds in t… ts. … eight of the peaks was record… s 587 … *…loids* extracted β-carotene, 442… carrot extra… β-carotene, and 13724 for sta… -carotene[29,30].

Figure 7. FT-IR spectra of β-carotene. (a) Standard, (b) extracted from Yeast and (c) extract from carrots.

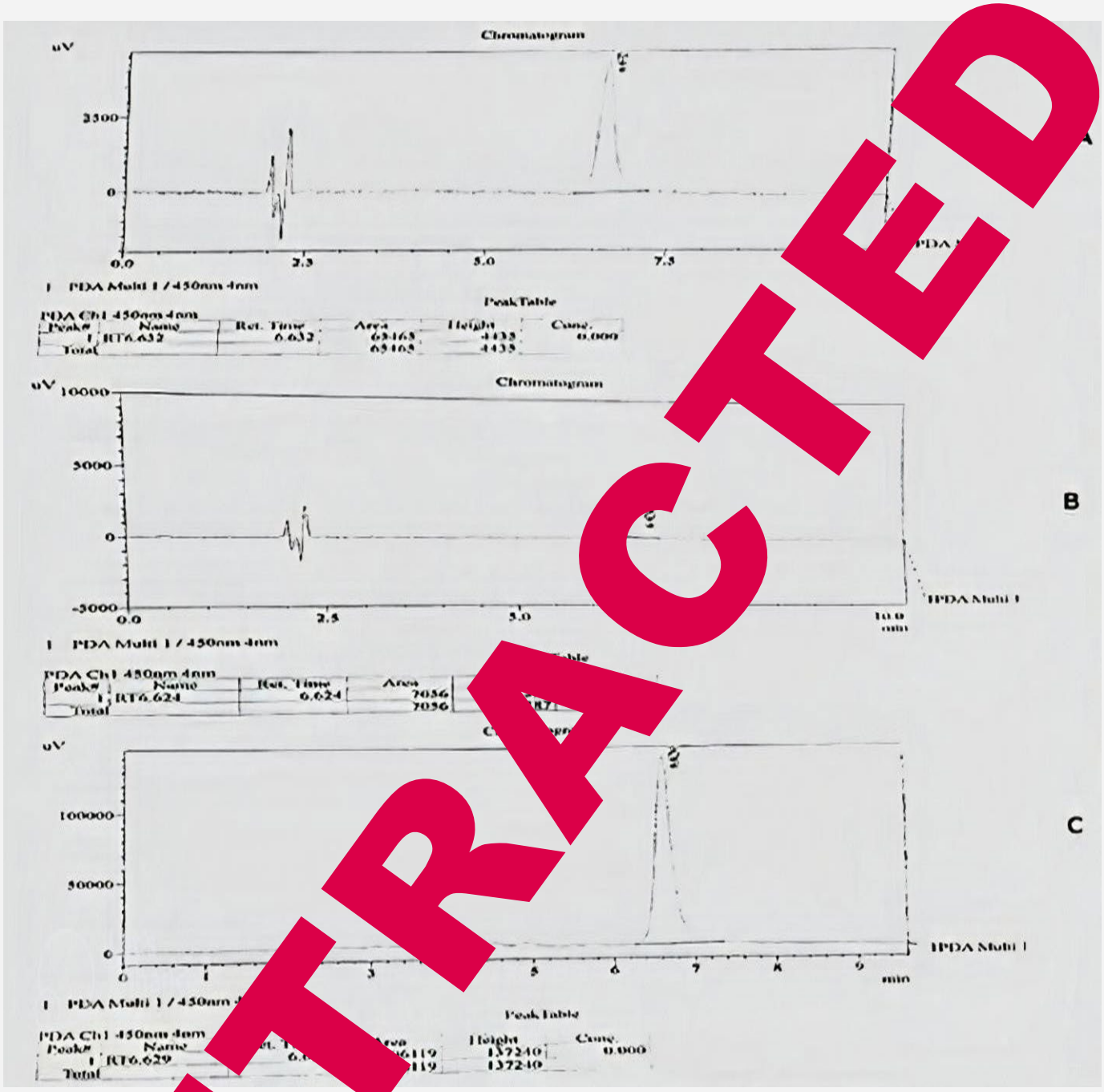


Figure 8. HPLC ysis (A) standard β-carotene (B) *R. toruloids* extracted β-carotene (C) carrot extracted β-carotene. The peak heights r enti e con entration of β-carotene, varied among the samples. The HPLC chromatograms displayed a peak height tanda 57 for *R. toruloids* extracted β-carotene, and 4435 for carrot extracted β-carotene. The difference in peak dica ariation in β-carotene concentration among samples.

Fu alysis

T funct ies, i.e., antimicrobial potential ti tial, were determined by the good di ethod and DPPH assay, respectively. Moreover, ation of yeast extracted β-carotene as a dye was a ecked.

The antimicrobial potential of extracted β-carotene was checked against one gram-positive (*Staphylococcus sp.*) and two gram-negative (*Salmonella sp.*, and *E. coli*) bacteria. For positive and negative control, standard β-carotene and dimethyl sulfoxide (DMSO) were used respectively. After 24 hours of incubation, zones of inhibition were recorded. *R. toruloids* extracted β-carotene remained highly effective against *Salmonella sp.*,

with the formation of (25.3±0.3 mm) zone of inhibition. For *Staphylococcus spp.* and E. coli, *R. toruloids* β-carotene produces inhibition zones of 19.8±0.3 and 19.6±0.5 mm respectively. The β-carotene extracted from carrots also showed the best antibacterial activity against Salmonella sp. as compared to *Staphylococcus spp.* and *E. coli*. However, the antibacterial potential of R. toruloids β-carotene was reported to be greater than carrots β-carotene, but less than the standard β-carotene as indicated in Figures 9 and 10.

The antioxidant activity of β-carotene extracted from *R. toruloids* and carrot was analyzed via DPPH assay. Upon addition of the antioxidant agent, a prominent shift in color from purple to yellow w in- dicative of the positive antioxidant tene (Figure 11a). The spectrosco alysis rev that the pigment had OD lesser th nk ind ing positive antioxidant activity (Figu y, the application of *R. toruloid* tracted β-c was determined by dying a pie cot in dilute cetone solution as devised by Ed . Fig re 12 shows the comparison of dye es by *ruloids* extracted β-carotene and ca cte arotene. The dyeing ability of *ruloids* β-carotene was reported simila at of carrot e ted β-carotene.

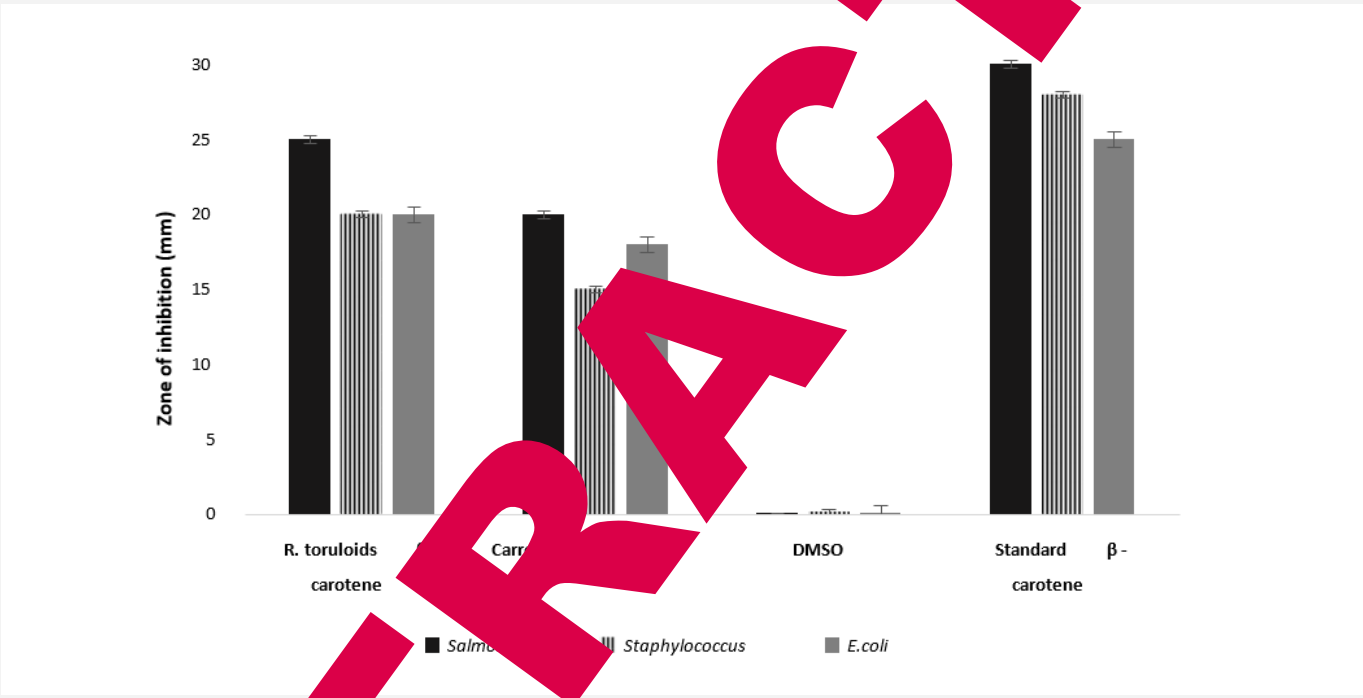


Figure 9. Antibacterial a y o e extracted from *R. toruloids* and from carrots in comparison with DMSO as negative control and standard β-car ene as a p ntrol against *Salmonella sp.*, *Staphylococcus sp.*, and *E. coli*.
*The error bars indi (±SD) of three re cates of each reading.

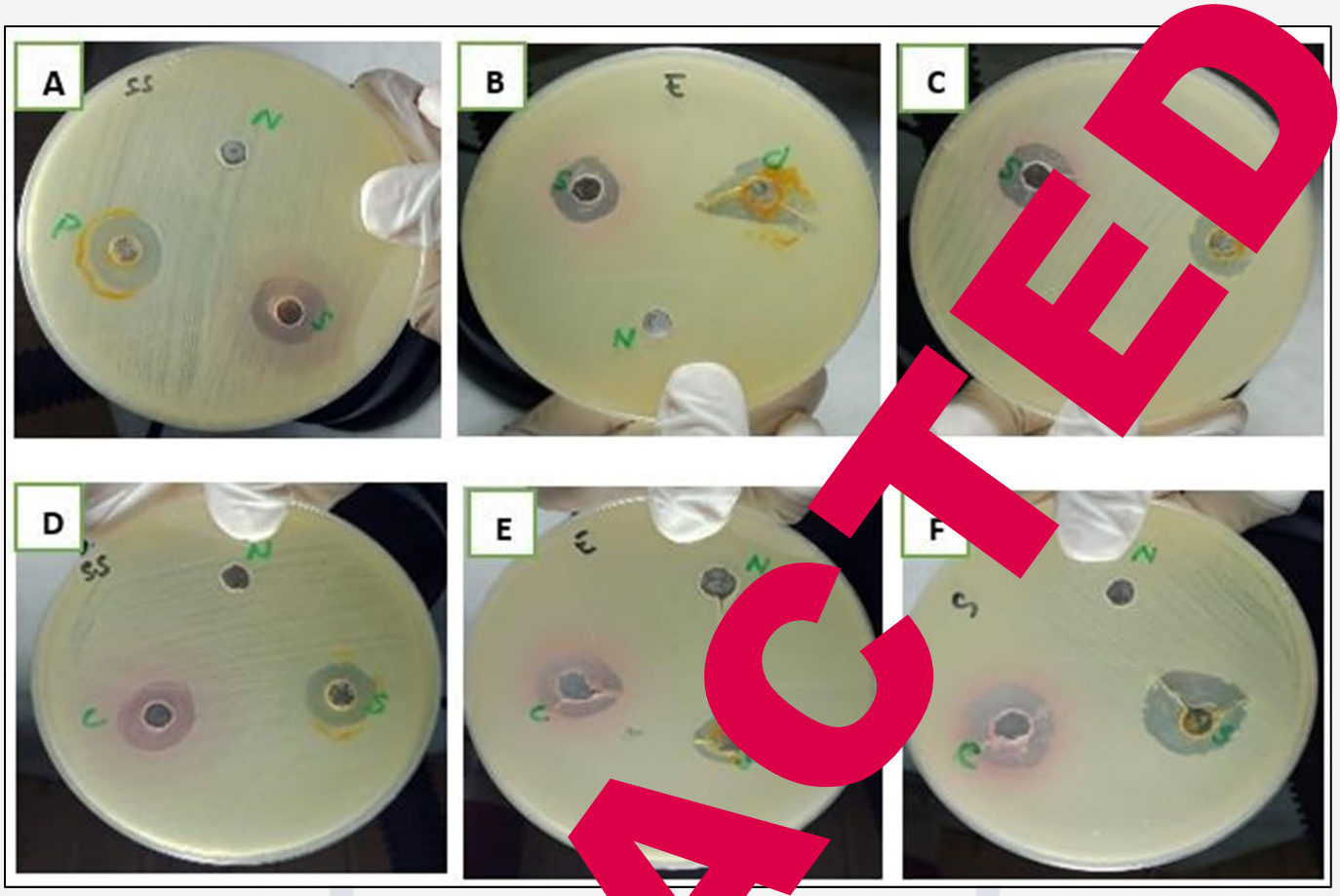
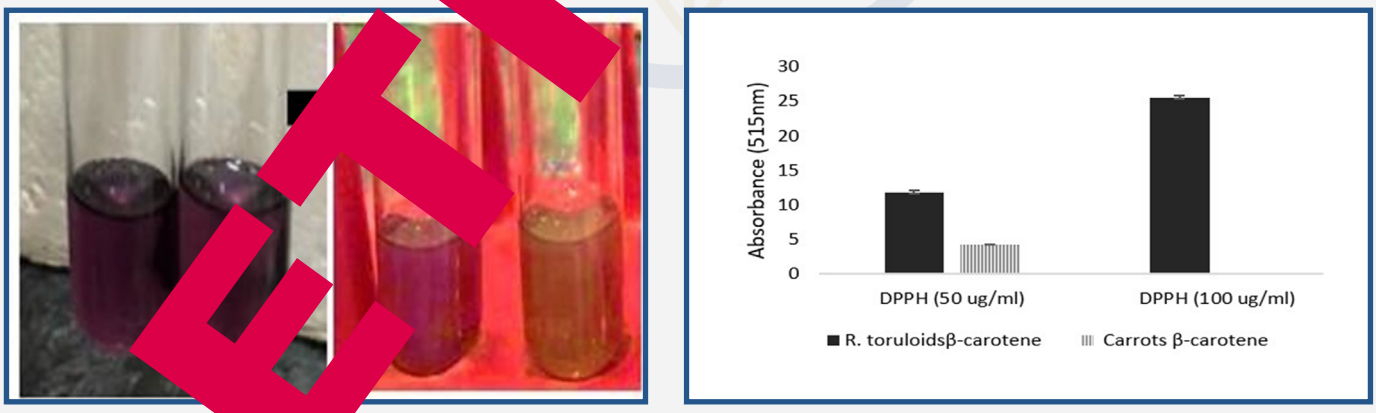


Figure 10. Inhibition zones of *R. toruloids* ex[illegible] β-carote[illegible] *inst* (A) *Salmonella sp.* (B) *E. coli* (C) *Staphylococcus sp.* and carrot extracted β-carotene against (D) [illegible] *sp.* (E) *E.* [illegible] (F) *Staphylococcus sp.*
*Here P or C= Standard β-carotene as p[illegible]e co[illegible] N= DMSO as negative control and S= sample as β-carotene extracted from *R. toruloids* and carrots, respecti[illegible]



(a) (b)

Fi[illegible] 11. [illegible]oxidant activity of *R. toruloids* extracted β-carotene and carrot extracted β-carotene (a) color change before and [illegible]the r[illegible] [illegible]orbance at 515 nm of DPPH assay.

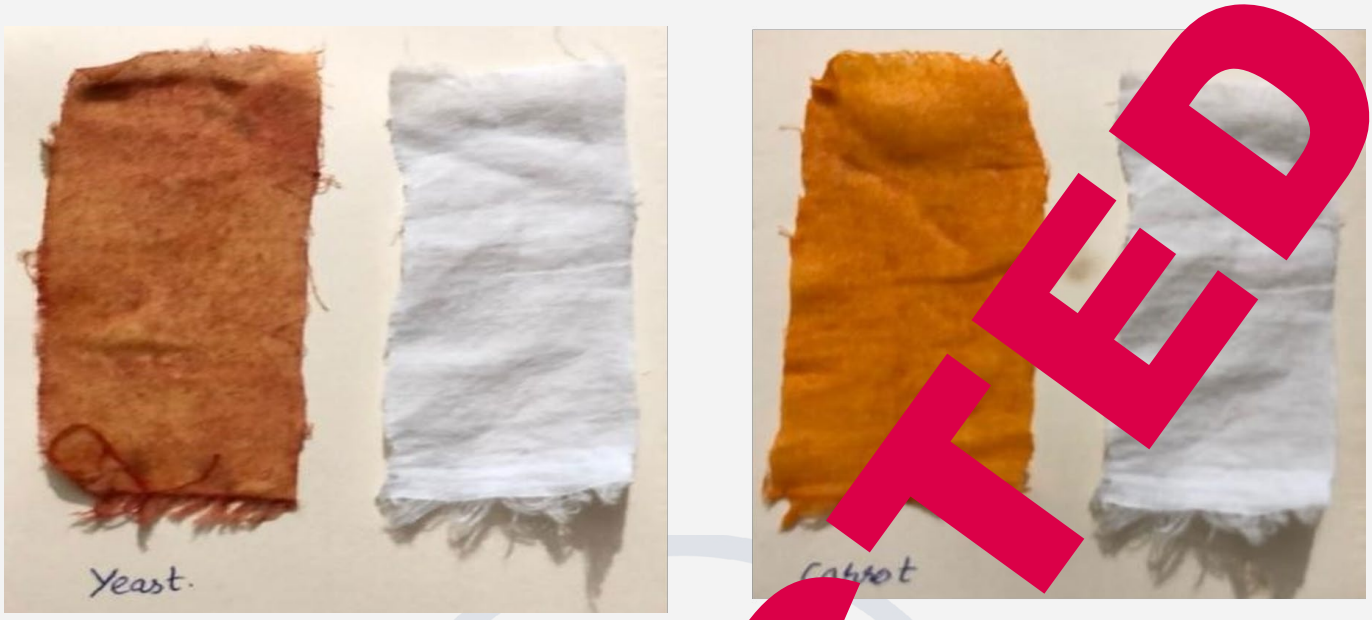


Figure 12. Application of β-carotene in dyeing cotton. (a) Cotton d with *R. tor extracted* β-carotene; (b) Cotton dyed with carrot extracted β-carotene.

DISCUSSION

Higher plants produce natural carotenoids in very s amounts due to their slow growth rates. Meanwhi microorganisms such as algae and yeast produce nat ural carotenoids in higher concentrations ng mi croorganisms, yeasts such as *Phaffia toru la sp.*, *Sporobolomyces sp.*, *Rhodos ium* and *Sporidiobolus sp.* efficiently produ ofus trations of β-carotene due to highe sity pro duction at less cost compared t algae resent study, pink-colored yeast co s were is om rotten orange peels. The p olor is suspecte to be due to the production ene, an orange-red dye[33]. Carbohydrates e ucers of β-caro tene production[32-34]; he e, bioch dentification of β-carotene-pro ing yeast strai as also con ducted on the h of carbohydrate assimilation test. The results i ted assimilation of glucose and dextrose wi p ction icating that the yeast strain has th y en s (zymase) and specif ic metabolic pat k down and utilize these sugar ther, the NA sequencing confirmed the n as *Rhodotorula toruloides*. Kim et al[35] a repo lation of β-carotene-producing lo rain, *Rhodosporidium babjevae*, i n citrus fruit. The difference in colony color is du ifference in strains.

After the tion and identification of *R. toruloides*, β-carotene production was conducted in different cul ture media (Basal, MS3, and YM). Figure 2a shows basal media (2% glucose, 0.4% yeast extract, 0.1% 0.05% $MgSO_4$) gave higher growth of yeast pared to the other culture media used, similar to the esults of Hu et al[36]. The presence of glucose provides n efficient energy source, yeast extract provides amino cids and peptides[37], while KH_2PO_4 and $MgSO_4$ main tain optimal ionic balance, which ultimately facilitat ed higher yeast production in 120 hours of incubation. Moreover, among different carbohydrate sources (glu cose, dextrose, and sucrose), YPD culture media with 1% dextrose induced maximum pigment production as indicated in Figure 2b. Gerelmaa et al[17] reported the fermentation of glucose, dextrose, and maltose by the yeast strain *Rhodotorula glutinis*. Saha et al[38] described that yeast produces secondary metabolites, mostly pig ments, in its stationary state. They also reported a high yield of β-carotene on YPD media till the end of the stationary phase of the yeast growth period. Kaur et al39 also indicated that *Blakeslea trispora* can grow in media containing fruits and vegetable waste as sub strate and gave a good yield of β-carotene, with respect to synthetic media containing all nutrient sources.

Optimization of different parameters is also important to achieve the specific conditions for maximal growth and pigment production. Response surface methodolo gy is considered a more efficient optimizing technique due to its systematic approach and ability to model and predict responses compared to conventional optimizing approaches[40]. The results of RSD indicated the optimal

conditions, i.e., pH 6.5, temperature 25°C with 4% sugar concentration, can give 2.8 mg/L production of β-carotene. Sharma and Ghoshal41 reported optimum conditions for β-carotene production were pH 6.1, and temperature 25.8°C. This minor variation in results is due to change in yeast species, and they used a bioreactor for fermentation, which provides more controlled conditions than a flask. Rodríguez et al[42] also reported 2968 µg/L (2.96 mg/L) β-carotene production by *Rhodotorula mucilaginosa* at pH 5, at 30°C using RSD. The slight change in parameters is due to the change in *Rhodotorula* strain. The ANOVA quadratic model indicated a significant interaction between temperature and pigment production. The standard plot showed that temperature had a significant impact on pigment yield with maximum production at 25°C. Zarandi-Miandoab et al43 also reported maximum pigment production at 25°C.

The characterization of extracted β-carotene was conducted via spectrophotometry, TLC, FTIR, and HPLC. The spectrophotometric analysis showed maximum absorbance at 460 nm and 490 nm in standard, carrot-extracted, and *R. toruloides-extracted* β-carotene. Karnjanawipagul et al44 and Nagaraj et al[45] reported similar results for β-carotene extracted from ca and *R. toruloides* CBS 14, respectively. The TL sults also gave similar Rf values in the range of 0.9 0.92, similar to that of standard β-carotene[46,47]. More over, the functional group analysis was co ucted on the basis of FTIR spectroscopy. The sp ained by FTIR confirmed the presence of (s n between 3000 cm^{-1} to 2500 cm^{-1}) ar =C–H between 1629 cm^{-1} to 1713 cm^{-1}), tional groups in β-carotene also report ivedi et al28. The HPLC results co d the e of β-carotene because the re n time peaks car rot-extracted and *R. to extracted* β-carotene were 6.629 and 6.624 s that of standard β-carotene as reported Rashid d Grigoryan et al29. However, th resence of nois carrot- and *R. toruloides-extra* β-carotene respectively indicated the presence o ne c metabolites along with the β-carotene.

After the ch ion o arotene, its biological activities, such a l and antioxidant activity, al ith its d lity, were analyzed. Both the nd *R. to oides-extracted* β-carotenes sh ed th ghest antibacterial activity for *Salmonel-*K lso reported that β-carotene has h icrobial activity against *Salmonella* and *E. coli*. er, the DPPH assay indicated that both carrot- a *toruloides-extracted* β-carotenes have greater antioxidant activity than the carrot-extracted β-carotenes. The DPPH assay is based on the measurement of scavenging activity of antiox d it. The starting point is violet color, wh educ e presence of antioxidant molecules loses its c The valence electron of the nitrog in DPP actually reduced by gaining a hydroge e antioxidant to the corresp g hydrazi uller and Böhm[49] described tha caro e exhibits ts antioxidant activity beca of speci chemical structure, which facili ions biological membranes, helping it t ant ant activity. Jaber and Majeed o repor arotene shows high antioxida tivity up to . Basically, the Z-isomers pr β-carotene are responsible for its antioxidant

Finally, th eing β-carotene was evaluated by dyeing a piece of co When cotton is immersed in β- e solution, the pigment molecules adhere to th fibers via weak bonds. The dyeing ability of otene is h se of the conjugated double bonds, h absorb l in the visible spectrum resulting in i cteris ange color[51]. The similarity of dyeing *toruloides-extracted* β-carotenes with the carrot xtracted β-carotene is very significant as it ows its potential application in the textile and food

CONCLUSIONS

In recent years, the demand for natural dyes has increased in the food, cosmetics, and textile industries because of their significance over synthetic dyes. Several plants, fruits, algae, and fungi are natural sources of pigments. Plants are a rich source of pigment, but their slow growth makes them a less preferable source. In this study, β-carotene an orange red pigment was produced from yeast Rhodotorula toruloids and its biological activities were evaluated and compared with carrot extracted β-carotene. The results of the study indicate that β-carotene extracted from R. toruloids showed better antimicrobial and antioxidative potential along with its efficient ability to dye cotton as compared to the carrots β-carotene. Hence, R. toruloids, being easily available and cost-effective production can prove better β-carotene source as compared to carrots.

Ethics Approval
Not applicable.

Consent for publication
This manuscript has not been published and is not being considered for submission to another journal for publication.

Data availability
Will be available on request.

Declaration of competing interest
The author declares no conflict of interest.

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