Isolation and identification of carotenoid producing yeast and evaluation of antimalarial activity of the extracted carotenoid(s) against *P. falciparum*

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Research

Abstract

Background: Malaria is a big threat and one of the major diseases and important cause of human illness and death in developing countries, in which majority of deaths were found to be due to Plasmodium falciparum malaria. It is becoming a serious challenge to fight against malaria because of the emergence of Plasmodium falciparum strains resistant to all anti-malarial drugs. As microbial carotenoids are well-known anti-oxidants, performing critical functions in plants and animals, in this study, the extracted microbial carotenoids were tested for their anti-malarial activity. Methods: To determine the anti-malarial activity of microbial carotenoids, five isolated yeast strains were identified, and characterized for their carotenoid production. The evaluation of anti-malarial activity of extracted carotenoids was tested on Plasmodium falciparum strain 3D7 using in vitro growth inhibition assay. Data was analyzed by FACS (Fluorescence activated cell sorter) and counted via gold standard Geimsa-stained smears. Extracted yeast carotenoids showed a profound inhibitory effect at a certain concentration when compared to β-carotenoid as control. Results: Yeast carotenoids showed a profound inhibitory effect at a concentration of $10\text{-}3$ µg/µl and $10\text{-}4$ µg/µl when compared to β- carotenoid as control. SYBR Green1 fluorescent dye was used to confirm the decrease in parasitemia at given range of concentration. Egress assay results suggested that treated parasite remained stalled at schizont stage with constricted morphology and were darkly stained. Non-toxicity of carotenoids on erythrocytes and on human liver hepatocellular carcinoma cells (HepG2 cells) was shown at a given concentration. Conclusions: Anti-oxidant and anti-carcinogenic activity of carotenoids is well established, so we embarked upon predicting anti-parasitic effects of yeast carotenoids to fight the dreaded disease, malaria. Results suggest that the extracted yeast carotenoids was able to kill the parasite by 98.3% and it was non-toxic to RBCs and HepG2 cells. This is the first report which provides strong evidence for anti-malarial effects of extracted yeast-carotenoids, which can be produced via a sustainable and cost-effective strategy and may be scaled up for industrial application.
Keywords: Carotenoids, yeast, Rhodotorula, β carotene, Plasmodium falciparum, malaria

Background

Malaria is a big threat and one of the major diseases in developing countries causing an estimation of 219 million cases of malaria occurred worldwide in 2017 [1]. In 2015, The World Health Organization (WHO) reported, that the huge majority of deaths, about 99% were found to be due to *Plasmodium falciparum* malaria [2]. Throughout the history of mankind, this extremely infectious disease has been one amongst the most important causes of human illness and death. *P. falciparum* is the most potent and prevalent infectious malarial species in tropical and subtropical countries due to the resistance of the parasite to a large number of the available antimalarial drugs [3]. The darker side of this disease is parasite resistance to the natural product artemisinin and chloroquine, the standard antimalarial drug used during decades [4].

It is becoming a serious challenge to fight against malaria because of the emergence of *P. falciparum* strains resistant to all anti-malarial drugs. Therefore, there is an urgent need to identify some new compounds which shows anti-malarial activity [5]. A promising strategy for research into new drugs and

moreover, to overcome drug resistance, is compounds derived from natural sources [6]. Natural resources are very important sources of potential drugs for treating various infections, and microorganisms are a rich natural source of diverse compounds [7]. Natural products including medicinal plants as well as microbial pigments may offer relatively cheap alternative treatment opportunities for malaria patients. [8,9]. Carotenoids are one of the natural pigments. These are ubiquitously found in nature and are synthesized by plants and microorganisms, in response to various environmental conditions, whereas animals have to obtain them from food [10]. Its colour and anti-oxidant properties, makes it exceptionally appealing in food and feed industry along with pharmaceutical and cosmetic industry [11]. Carotenoids, especially β-carotene, act as antioxidants by reacting with active oxygen species [12] and also as anti-carcinogenic agents [13]. Anti-oxidant activity of carotenoids is well established [14, 15], so we embarked upon predicting anti-parasitic effects of yeast carotenoids to fight the dreaded disease, malaria. This study is the first report demonstrating anti-malarial activity of yeast carotenoids, which showed immediate effect upon treatment, by changing the cell morphology leading to death at later stages. In the present study, we aimed at isolation and identification of carotenoids producing yeast strains from various sources, extraction and separation of carotenoids and determining the effect of extracted carotenoids on *P. falciparum*.

Methods

Isolation of carotenoid producing yeast strains

To isolate carotenoid-producing yeast strains, the isolation source selected was soil/ compost, peels, flower petals, etc. About 1g of the above material were added to different flasks containing isolation medium (10ml) and incubated for 24-48h. After growing for 48h, the medium was serially diluted from 10^{-1} to 10^{-10}. 0.1 ml of the each dilution was spread on yeast extract peptone dextrose agar (YPD) plates. Best colony formation was observed in 10^{-6} dilution spread plate. Following 2-4 days of incubation at room temperature, morphologically different isolates were selected and transferred to fresh YPD plates and stored appropriately at 4°C and at -80°C as glycerol stocks.

Identification and characterization of selected yeast strains

Selective yeast strains Y1, ab1, WEP, CS1 and CS2 were characterized, first on the basis of morphology, followed by rDNA sequencing. Phenotypic characterization was done on the basis of colony and cell morphology after visualizing them under Nikon Eclipse Ni Microscope (NIS Elements).

Genomic DNA isolation and rDNA sequencing

Yeast genomic DNA isolation

Total yeast DNA was extracted by standardized method of Guillamon et al., 1994 [16]. Yeast colonies were picked from YPD medium selective plates after two days of growth at 30°C. Alternatively, single colonies were picked from plates and inoculated in 5 ml Yeast Peptone Dextrose (YPD), and grown at

30°C for approximately 24h. About 2ml of the overnight cultures were centrifuged at 1,254× g for 5 min and the cell pellets obtained, were re-suspended in 1 ml 1X PBS buffer and again centrifuged at 2,822× g for 4 min. The pellet was re-suspended in 0.5 ml of TE buffer [50 mMTris-HCl (pH7.4), 20 mM EDTA]; the mixture was then incubated for 30 min at 65° C. Followed by addition of glass beads and P:C:I (Phenol:Chloroform:Isoamyl alcohol) solution to the mixture, which was placed on ice and centrifuged for 5 min. The supernatant was transferred to a fresh tube, mixed with an equal volume of 2-propanol, and incubated for 5 min at room temperature. The pellet was air-dried and dissolved in sterile distilled water.

PCR amplification

The 5.8S-internally transcribed spacer (ITS) rDNA regions (ITS1 and ITS2) of all strains were PCR amplified from the genomic DNA using the ITS1 and ITS4 primers. PCR amplifications were performed in a 50 μL reaction volume supplemented with 50 ng of genomic DNA, 10pmol of each primer, 10 mM of each dNTP and 2.5 units of Taq DNA Polymerase (G-Biosciences, USA). All the amplifications were programmed in a T100 thermo cycler (BIO RAD, India) as follows: 95°C for 3 min, followed by 30 cycles of 95°C for 30 sec, 52°C for 30 sec and 72° C for 1 min, with a final extension step at 72° C for 10 min. The amplified PCR products were separated on a 0.8% (w/v) agarose gel in 1X TAE (40 mMTris-Acetate, 1 mM EDTA (pH 8.0) buffer and detected by staining with ethidium bromide. The amplified PCR products of the 5.8S rDNA regions were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced using the ITS1 and ITS4 primers. The sequences were submitted to NCBI to get the accession number.

Carotenoid extraction and determination

About 5 ml of 48-h-old seed culture (log phase) was used to inoculate 100 ml of growth media and incubated in an orbital shaker at 120 rpm. Samples were collected at every 6-12 h interval for determination of OD (600 nm), dry and wet cell weight, total sugar and reducing sugar of the medium as well as carotenoid content [17]. Carotenoids were extracted by a modified method of Kim et al., 2004 [18]. After centrifugation, the cells were collected, washed and treated with DMSO at 50°C for 1h and finally transferred to the hexane phase by liquid-liquid extraction. The OD of the supernatant was determined at 450 nm against hexane using a Systronics UV-Vis spectrophotometer. Quantification was done using standard curve of β-carotene (prepared in hexane). Standard β-carotene was obtained from SRL PVT Ltd, India. Total carotenoid content was expressed as the content of β-carotene that was determined spectrophotometrically at 475nm. All statistical calculations (t-Test) were done using MEDCALC Software bvba statistical software (www.medcalc.org) for determination of p-value.

Thin Layer Chromatography (TLC) analysis for the separation of the pigment fractions

Thin Layer Chromatographic separation of the different fractions from the crude carotenoid pigment of all isolated yeast strains was carried out using silica gel 60 TLC plates (Merck, Germany) using petroleum ether and acetone (80:20, v/v) as a mobile phase and determined their R_f values. The samples were identified by comparing the distance travelled by the standard to the distance travelled by the test sample

β-carotene. The R_f values is a mathematical representation of the ration of the distance travelled by the solvent [19]. Perrier et al. (1995), reported that β-carotene was the major pigment (78.6%) present in the *Rhodotorula* strain [20]. The isolated yeast strain, *Rhodotorula glutinis* ab1 produced good amount of carotenoids and shows different bands in TLC analysis. So we chose the carotenoid extract of *Rhodotorula glutinis* ab1 strain for further study.

Preparation of stock and working concentrations of carotenoids

Standard β-carotene (SRL Pvt Ltd, India) and carotenoid extract were resuspended in dimethyl sulphoxide (DMSO) for preparation of 1 mg/ml and 2.5 mg/ml stock solution respectively. Further 0.1 mg/ml working stocks were prepared and eventually used to set up the various assays. Negative controls contained parasites in solvent without treatment.

In-vitro *P. falciparum* culture

Parasites were cultured at 2.5% hematocrit in RPMI-HEPES medium at pH 7.4 supplemented with hypoxanthine at 50 μg/ml, $NaHCO_3$ at 25 mM, gentamicin at 2.5 μg/ml and Albumax II (Gibco) at 0.5% (w/v). To obtain enriched ring stage parasites, a standard sorbitol synchronization method was used. *P. falciparum* strain 3D7 was used for all experiments.

Microscopic examination of *P. falciparum*

Thin smears of *P. falciparum* cultures were prepared on glass slides, fixed with 100% methanol, and stained with fresh Giemsa (Sigma) solution made in filtered distilled water. Smears were examined with a 100X oil immersion objective and a standard phase-contrast microscope (Leica). Images from the smears were captured with Nikon camera and processed with Adobe Photoshop.

Growth inhibition assay by FACS

In vitro growth inhibitory activities of carotenoid extract and standard β-carotene against strain 3D7 were evaluated at concentrations ranging between 10^{-3}μg/μl–10^{-5}μg/μl for standard β-carotene and between 10^{-3}μg/μl–10^{-6}μg/μl for carotenoid extract respectively. Around 34–38h of sorbitol synchronized cells of strain 3D7 at a late trophozoite-stage with an initial parasitaemia of 0.8%, at 2% haematocrit, were incubated with the compounds at above-mentioned concentration over one intra erythrocytic cycle of parasite growth; untreated parasites served as control. Following a 48h incubation, the whole sample was collected and washed twice with 0.01M PBS and stained with ethidium bromide (10μM) (Sigma Aldrich, USA) for 15 min at room temperature in dark. The cells were washed with 0.01M PBS, and analysed by flow cytometry on ARIAIII (Becton Dickinson) using Flow-Jo software. Fluorescence signal (PE) was detected with the 590 nm band pass filter using an excitation laser of 488 nm collecting 100,000 cells per sample. Following acquisition, parasitaemia was estimated by quantifying number of infected erythrocytes which were determined by the proportion of PE-positive cells using Flow-Jo software.

Progression assay

Tightly synchronized, ring stage culture (10-12 hours) was diluted to 1% parasitemia & 2% hematocrit to determine any delay in progression upon treatment. Briefly, each well with 1% parasitemia and 2% hematocrit was treated with 1 μg/ml and 0.1μg/ml of standard β- carotene as well as carotenoid extract for period of 48 hours. Untreated cells served as control. To evaluate any morphological and progression variation, Giemsa stained smears were prepared at respective time points 24, 36, 48 and 56 hours. Nearly 2000 cells were scored by light microscopy.

Detection of parasitaemia using SYBR Green1 fluorescence

*P. falciparum*3D7 synchronized culture at late trophozoite stage with initial parasitaemia of 5% was diluted to 0.8% and packed erythrocytes were added to maintain 2% haematocrit in each well of 96-well microtitre plate keeping total volume of 100μl per well. The wells were treated with above mentioned concentration of carotenoids and incubated for 48h. Further, after completion of one intraerythrocytic cycle 100 μl of lysis buffer [20 mMTris (pH 7.5), 5 mM EDTA, 0.008 % (W/V) saponin, and 0.08 % (V/V) Triton X-100] containing Syber Green (1× final concentration) was added in each well. The plate was then incubated in the dark for 2h, fluorescence was measured at 485 nm (excitation) and 528 nm (emission). Parasite level was then plotted against fluorescence intensity.

Monitoring of the *P. falciparum* egress phenotype

To evaluate the egress of parasite from erythrocytes Egress assay was performed as mentioned by Garg et al., 2013 [21]. Tightly synchronized schizont stage parasites (~44-46 hpi) were enriched using 65% percoll with 85-90 % purity. Parasite culture was diluted at a final parasitaemia of 10% schizonts in 2% hematocrit. These were then seeded in a 96 well flat bottom plate (100 μl /well) and were exposed to respective treatments of carotenoids for 7-8h. BAPTA-AM was taken as positive control of egress inhibition in the study. After 8h the thin blood smears were made on glass slides and were stained with Giemsa stain for microscopic analysis.

Parasite egress % was calculated as the percent of schizonts ruptured in treatment and control during the incubation time as compared with the initial number of parasites at 0h, using the formula described below.

$$parasite egress(\%) = \left(\frac{I-T}{I-C}\right)x100$$

I, % of schizonts before treatment; T, % of schizonts after treatment with inhibitors; C, % of schizont after treatment with solvent control. Parasite egress % was then plotted considering percent of schizonts ruptured in control as 100% egress.

Hemolysis Assay

Hemolysis assay was performed as described by Lale et al., 2015 [22] with little modification. 3 ml of human blood was centrifuged at 176×g for 10 min. Red blood corpuscles (RBC) pellet was washed thrice with 0.01M PBS and diluted to 6ml to prepare RBC stock solution. Various concentrations of carotenoids ranging from 10^{-1} μg/μl – 10^{-6} μg/μl were added and incubated with RBC stock solution (300 μl each) at 37°C for 3h. Post incubation, supernatant was collected and centrifuged at 490×g for 5 min. 100 μl of hemoglobin released in the supernatant was analyzed at range of wavelength (400-420 nm) using UV–visible Multimode Reader (Varioskan™ Flash, Thermo Scientific, Singapore). Percent hemolysis was estimated with respect to hemolysis caused by negative control (PBS) and positive control (De-ionized water), as given by the following equation:

$$Hemolysis(\%) = \left(\frac{Sample_{400-420\,nm} - NegativeControl_{400-420\,nm}}{PositiveControl_{400-420\,nm} - NegativeControl_{400-420\,nm}} \right) x100$$

Cell viability Assay

Human liver hepato cellular carcinoma cells (HepG2 cells) were seeded in 96-well plates at a seeding density of 20,000 cells/well was allowed to adhere overnight at 37°C. Adhered cells were treated with Compounds at above mentioned concentrations and incubated for 24h. Untreated cells were maintained as positive control. Cytotoxic effects were assessed using MTT, conversion of MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to formazan crystals by viable cells was measured at 595 nm using spectrophotometer (Bio-Rad, USA).

$$Cellsurvival(\%) = \left(\frac{At - Ab}{Ac - Ab} \right) x100$$

At, Average absorbance of treated sample; *Ab*, Average absorbance of blank; C, Average absorbance of control.

Statistical Analysis

All of the experiments were performed in triplicates, and the standard deviation is reported with error bars. The analysis of variance method followed by a Student's t-test has been performed to determine the statistical significance. Differences were considered significant at $p < 0.05$.

Results

Isolation, selection and identification of yeast strains

After dilution plating on YPD agar, various pink colonies were obtained, which visually differed on the basis of colony morphology/ colour. Out of them, four different isolates were selected after viewing under the microscope at 100X magnification to observe their cell shape (Table 1). Apart from the isolated

strains, we collected one more yeast strain for this study, *Rhodotorula glutinis* Y1 (accession no. KY608223), from Indian Agricultural Research Institute, Pusa, New Delhi. This strain was isolated from rotten vegetables. *R. toruloides* ATCC204091 was used as reference strain for all the studies.

Identification by sequencing ITS regions 5.8S rDNA

Strains produced a single PCR amplicon of about 700 bp which was sequenced. On the basis of BLAST analysis of this sequence, the isolates were identified as *Rhodotorula glutinis* ab1, *Rhodotorula mucilaginosa* WEP, *Rhodotorula dairenensis* CS1, *Rhodotorula mucilaginosa* CS2 (Table 1). All the isolates belonged to the genus *Rhodotorula* but showed differences in cell shape or in their growth and carotenoid production.

TLC analysis of carotenoid pigment extracted from isolated yeast strains

In the present study, the pigments of isolated yeast compounds were separated using petroleum ether: acetone (80:20) as mobile phase. Different bands were separated from the pigment fractions (Fig 1). The R_f value of the extracted fraction (0.90) was similar as that of standard β-carotene spot and also there was a resemblance in their absorption spectra as well [23]. Close agreement was obtained between absorption maxima of these fractions which resembles with that fractions as, β-carotene, torulene and torularhodin was published earlier [24].

Anti malarial activity of extracted carotenoid

Antimalarial activity of carotenoids produced by *R. glutinis* against *Plasmodium falciparum* was investigated in our study using FACS analysis and Geimsa staining of thin smears (Fig. 2A i-x). The standard β-carotene showed its maximum inhibitory effect at a concentration of 0.001μg/μl (10^{-3}μg/μl) and minimum inhibitory effect at a concentration of 0.00001μg/μl (10^{-5}μg/μl). The carotenoid extract showed its maximum inhibitory effect at a concentration of 10^{-3}μg/μl and minimum inhibitory effect at 10^{-6}μg/μl. Out of various concentration of Standard β-carotene and carotenoid extract, 10^{-3}μg/μl and 10^{-4}μg/μl concentrations exhibited the best growth inhibition of 99% and 95% for β-carotene; 98.3% and 78.3% for carotenoid extract respectively (Fig. 2C). However, at β-carotene concentration of 10^{-5}μg/μl there was an increase in parasitaemia (Fig. 2B). Further using SYBR Green1 fluorescence dye we have confirmed the decrease in parasitaemia at given range of concentration used for performing the assay for both Standard β-carotene and carotenoid extract respectively (Fig. 2D).

Effects of tetraterpenoid on *P. falciparum* egress

Late schizont stage parasites (~44 to 46 hpi) were treated with optimal inhibitory concentrations of 10^{-3}μg/μl and 10^{-4}μg/μl for both β-carotene and carotenoid extract and allowed to develop until the appearance of ring stage parasite in untreated controls (~54 hpi, approximately 10 h post rupture) (Fig. 3). Parasitaemia was scored using microscopic examinations of Giemsa-stained thin smears. At above mentioned concentrations, there was a drastic inhibition of transition of schizonts to rings with ≥70%

efficiency compared to that of untreated control samples (Supplementary fig). This clearly suggested that treated parasite remained stalled at schizonts which showed constricted morphology and were darkly stained.

Effects of carotenoids on hemolysis of erythrocytes

To check the toxic effects of carotenoids on erythrocytes owing to their lytic phenomenon, RBCs were treated at a range of concentrations from high (10^{-1} µg/µl) to low (10^{-6} µg/µl). The values were measured spectrophotometrically keeping de-ionized water as positive control (Fig. 4 A-B). The data showed substantial % hemolysis in control with no or negligible lysis in treated erythrocytes, suggesting non-toxic effect of carotenoids on them.

Effect of carotenoids on mammalian cells

The effect of these molecules was also checked for their effect on mammalian cell viability. Carotenoids showed no cytotoxicity towards human liver hepatocellular carcinoma cells (HepG2 cells) at a concentration of 1µg/µl for 24 h (Fig. 4C).

This is the first report showing the anti-malarial activity of yeast-carotenoid that killed 98.3% parasite, where pure standard β-carotene showed 99% activity.

Discussion

Plant extracts, such as, quinine, obtained from *Cinchona* species, and artemisinin, obtained from *Artemisia annua* are well known antimalarial agents [25]. However, extraction of pigments from plant is laborious and often limited by the availability of the sources, dependent on seasons, geographical conditions etc. So, currently exploring microbial source for the production of pigments by fermentation is on demand. Bacterial prodigiosines, obtained from, bacterial strain, *Serratia marcescens*, such as, cycloprodigiosin, prodigiosin, undecylprodigiosin, heptyl prodigiosin, and metacyclo prodigiosin have demonstrated antimalarial activity against *P. falciparum* [26, 27, 28]. Similarly, bacterial pigment, violacein from and *C. violaceum*, and melanin produced by *Bacillus thuringiensis* subsp. *israelensis* respectively, exhibit anti-malarial activity in vitro against *Plasmodium falciparum* [29, 30, 31]. However, there is no report till date on the antimalarial activity of yeast carotenoids. Low production cost and promising potential against parasites has made yeast carotenoids very demanding. Yeasts of the genera *Rhodotorula* are able to synthesize carotenoid of high economic value and therefore represent a biotechnologically interesting group of yeasts. Our study shows the anti malarial activity of the extracted yeast carotenoid. Carotenoids extract of isolated yeast strain was tested for the anti-malarial activity against 3D7 strain of *P. falciparum* and cytotoxicity on human liver hepatocellular carcinoma cells (HepG2 cells).

Conclusion

The isolated yeast strains belonging to the genus *Rhodotorula* were able to synthesize carotenoids having high economic value and can be cultivated in synthetic media. The extracted carotenoid was non-toxic to RBCs and HepG2 cells, but active against malaria parasite 3D7 strain of *P. falciparum.* Results suggest that the extracted carotenoid from *R. glutinis* ab1 strain was able to kill the parasite by 98.3% as compared to standard β-carotene, in which the percentage of killing was 99.58%.

List Of Abbreviations

R.glutinis: Rhodotorula glutinis; OD: Optimal density; DCW: Dry cell weight; YPD: Yeast extract, Peptone, Dextrose; PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid; TAE: Tris acetate EDTA; *P. falciparum*: *Plasmodium falciparum*; DMSO: Dimethyl sulpho-oxide; RBCs: Red blood cells; FACS: Flourescence activated cell sorter; NaHCO$_3$: Sodium bicarbonate

Declarations

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Authors' contributions

All authors contributed jointly to all the aspects of the work reported in the manuscript. SS and GS performed the experiments and data analysis at the Amity University, Noida (India). Malaria parasite based experiments were done by AC at Shiv Nadar University. DNA isolation part was done with the help of KK and NG at ICGEB, New Delhi. DP and SS supervised all experimental work and data analysis. All authors have read and approved the final manuscript.

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Availability of data and materials

The data generated or analysed during the current study are included in this article.

Ethics approval and consent to participate

This study was approved by IBSC-JNU, New Delhi, India.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.
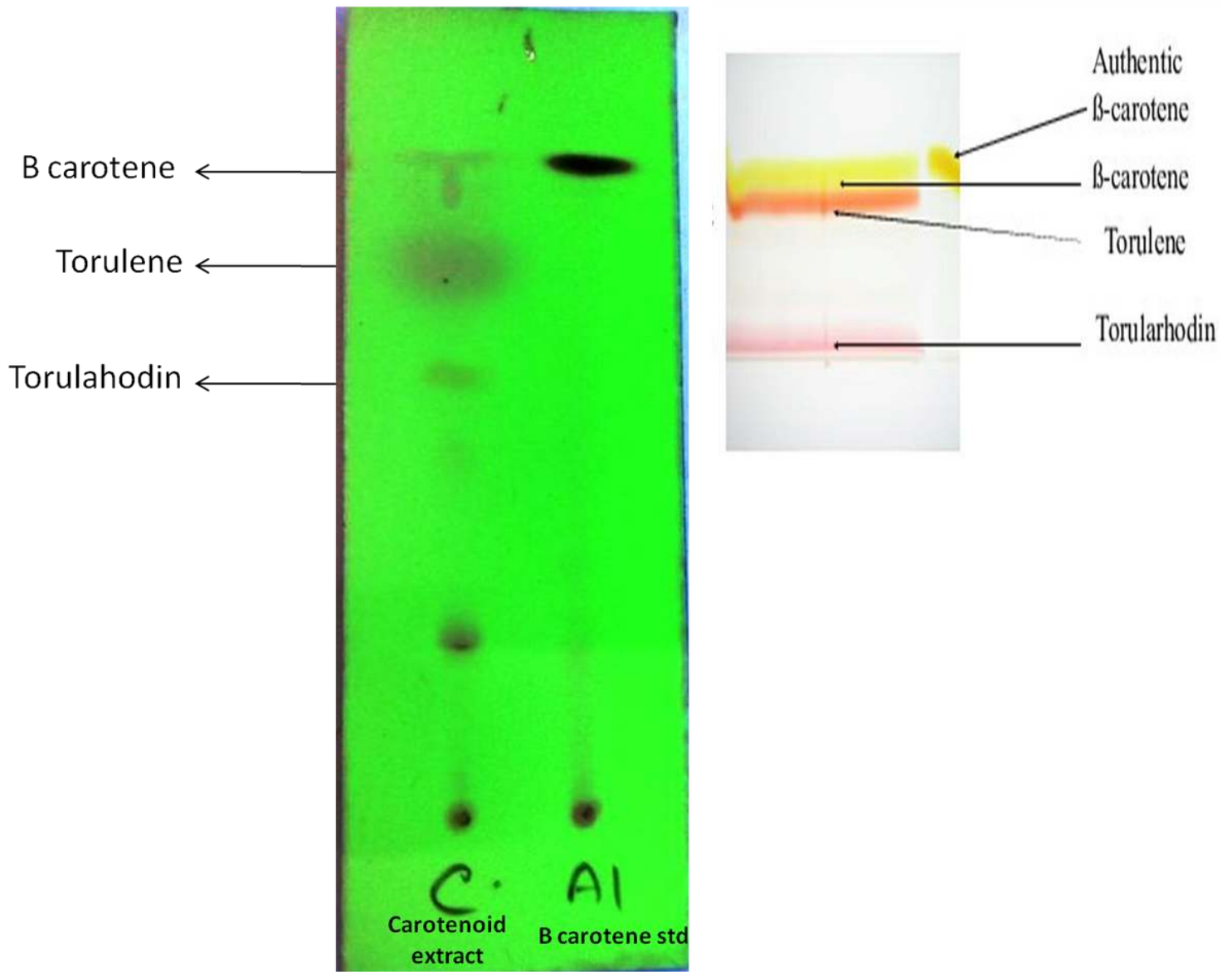
Figures

Figure 1

Comparision of TLC of carotenoid pigment from different sources with our isolated yeast strains ; (A) TLC of carotenoid extract from isolated yeast strains and sequentially developed with solvent system, petroleum ether-acetone (80:20) and (B) Thin layer chromatography (TLC) of separated carotenoid pigments from R. glutinis on silica gel plates 60A (20x20 cm)
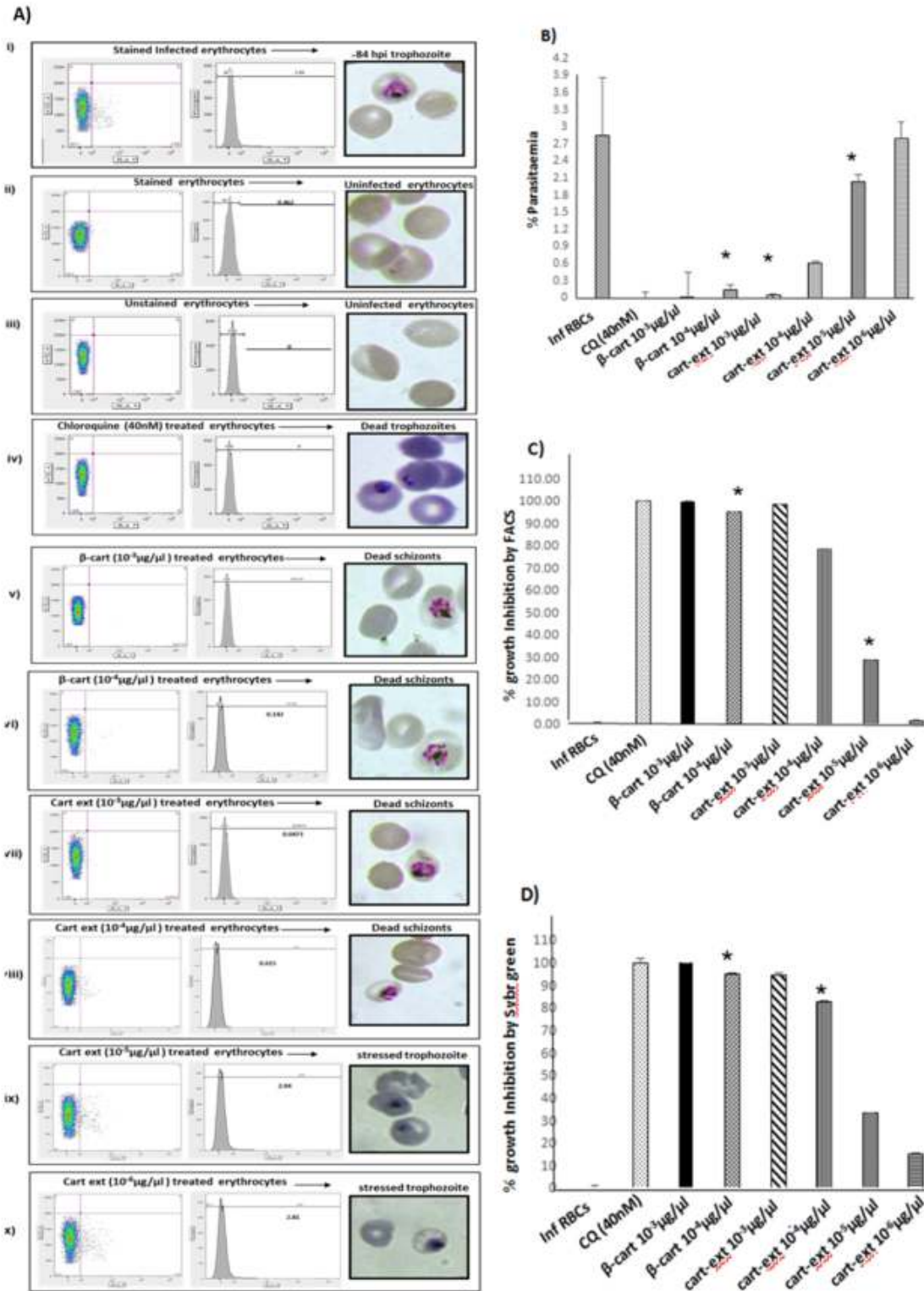
Figure 2

Cytotoxic effect of β- carotene and carotenoid extract on growth of P. falciparum in vitro (A i-x) Growth inhibition assay was performed with 36h post –invasion (hpi) trophozoites. After completion of 48h, the infected RBCs and uninfected controls were stained with EtBr for 15mins and washed with incomplete RPMI 1640 medium, and intensity of fluorescence was determined by flow cytometer with an excitation filter at 488 nm and emission filter at 595 nm along with light microscopy images of parasites at post

48h. At 84 hpi healthy new trophozoites were observed in solvent control whereas they were >80% reduced in the presence of standard β- carotene and carotenoid extract (at 10-3µg/µl) respectively. The parasite was stalled as late trophozoites/schizonts at further concentration range. Chloroquine was used as positive control, (B) Bar graph depicts percentage decrease in parasitaemia in treated erythrocytes as compared to solvent control post 48h, (C) Graph showing percent inhibition in growth of parasite when treated with standard β- carotene and carotenoid extract in dose dependent manner and (D) Spectrophotometer based SYBR Green assay showing percent inhibition of growth of parasite. Fluorescence was measured at 485 nm (excitation) and 528 nm (emission)
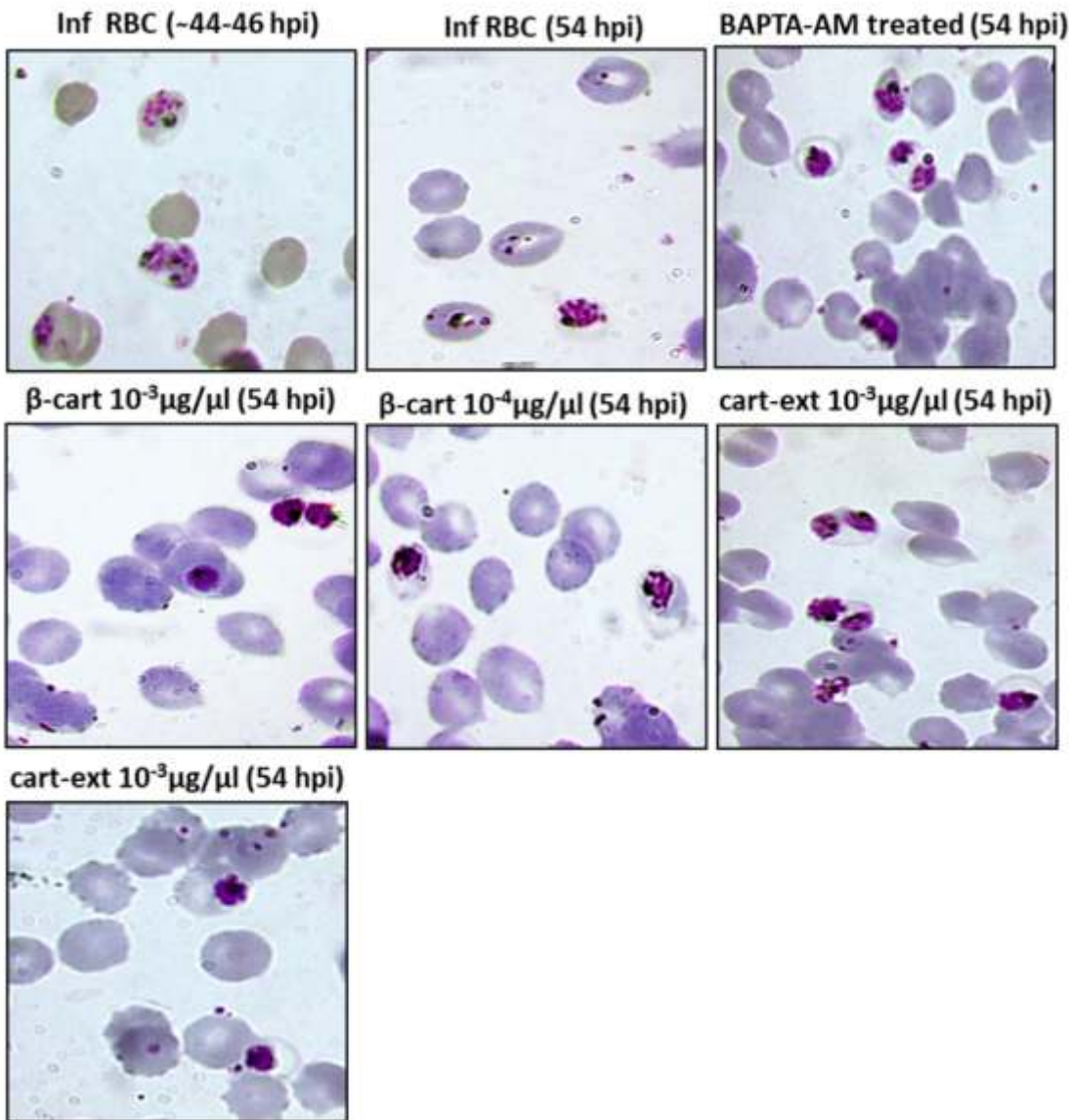

Figure 3

Effect of carotenoids at different concentration on parasite egress: Late-stage schizonts (~44-46 hpi) At a final haematocrit of 2% and parasitaemia of 10% are seeded in duplicate in 96-well flat-bottom plates and treated with carotenoids with range of concentrations. Solvent-treated parasites are used as control and BAPTA-AM treated parasites are used as a positive control to monitor unruptured schizonts.
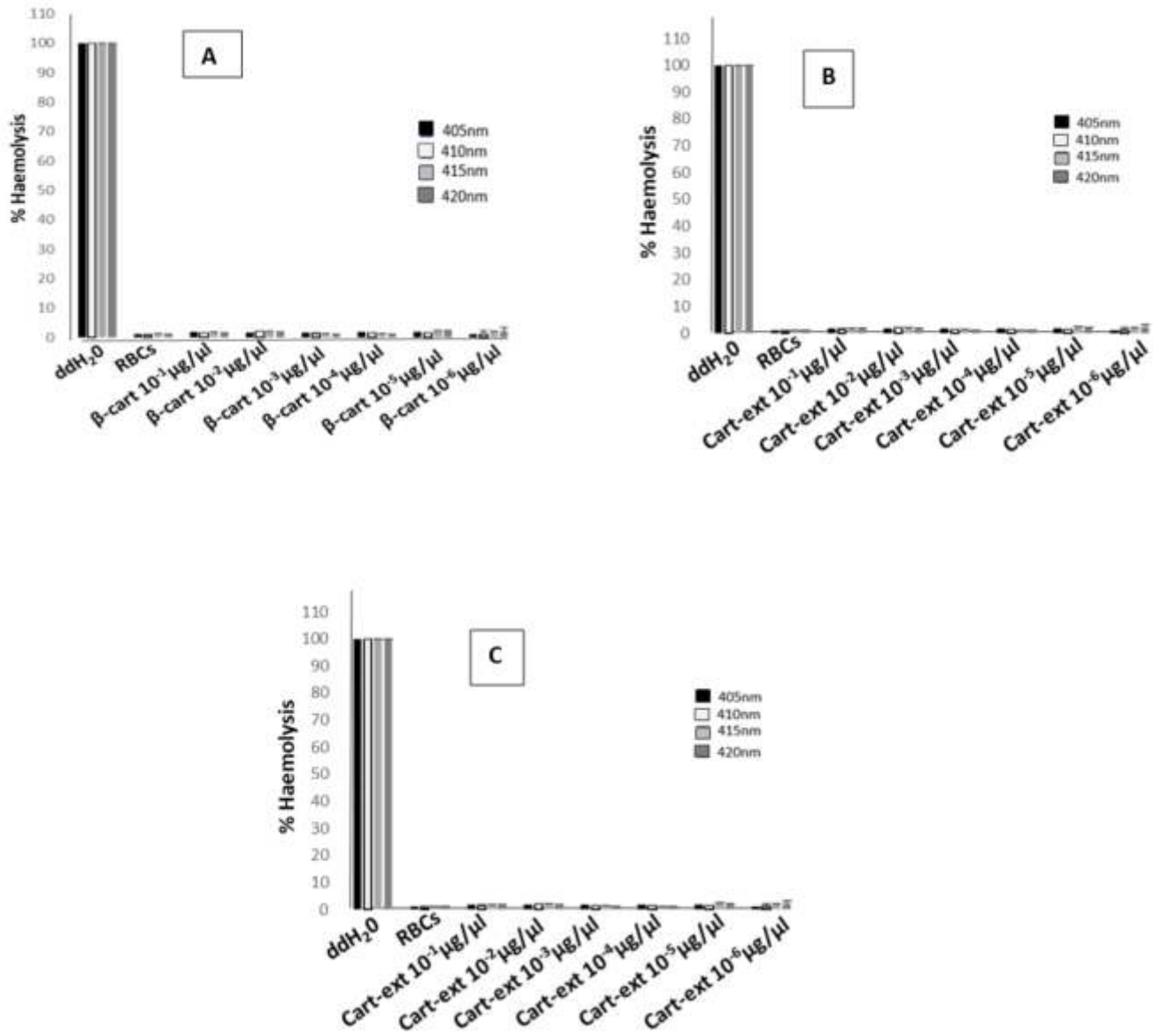
Figure 4

Effect of carotenoids on (A-B) haemolysis of erythrocytes and (C) on cellular viability (A-B) Post 3h incubation of RBCs at various concentrations of carotenoids ranging from 10 -1 µg/µl − 10 -6 µg/µl haemoglobin released in the supernatant was analysed at range of wavelength (400-420 nm) using UV−visible Multimode Reader. Percent haemolysis was estimated with respect to haemolysis caused by negative control (PBS) and positive control (De-ionized water). C) Effect of carotenoids on HepG2 cells viability. The graph shows the percent survival of cells in the presence of the standard and extract. Cell viability was measured by MTT assay after treatment withdose dependent concentration of carotenoids for 24h.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile.docx
- Table1.pdf