J Food Sci Technol (August 2017) 54(9):2728–2736
DOI 10.1007/s13197-017-2709-2

ORIGINAL ARTICLE

# Determination of antioxidant activity of *Hibiscus sabdariffa* and *Croton caudatus* in *Saccharomyces cerevisiae* model system

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Revised: 10 May 2017 / Accepted: 16 May 2017 / Published online: 9 June 2017


**Abstract** From ancient times, plants and plant derived products are exploited as a prominent source of folkloric medicines with tremendous therapeutic potential for an array of health disorders. In the present study, ethanolic leaf extract of *Hibiscus sabdariffa* and *Croton caudatus* were evaluated for free radical scavenging activity in *Saccharomyces cerevisiae* model system. *H. sabdariffa* and *C. caudatus* showed tremendous DPPH free radical scavenging potential with an $IC_{50}$ value of 184.88 and 305.39 µg/mL respectively at a concentration of 500 µg/mL. The ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* also showed significant hydoxyl radical scavenging and total antioxidant activity. Ascorbic acid was used as positive control. The in vitro antioxidant activity was further supported by in vivo studies using radical scavenging mechanism in *S. cerevisiae* wild type and its isogenic deletion strains *sod1Δ* and *tsa1Δ*. The mutant yeast cells substantially scavenged the stress generated by $H_2O_2$ when supplemented with ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* as evident from spot assays followed by fluorescence assay (DCF-DA) using fluorescence microscopic and intensity studies. *H. sabdariffa* and *C.caudatus* significantly neutralize the ROS level in yeast mutants with concomitant decrease in fluorescence intensity as compared to the untreated yeast cells. The results suggested the efficacy of *H. sabdariffa* and *C. caudatus* as potent antioxidants in yeast system and thus their futuristic applications in therapeutics.

**Keywords** Antioxidant activity · DPPH · Dichlorofluorescin · *Saccharomyces cerevisiae* · Reactive oxygen species

## Introduction

In living organisms free radicals are invariably generated as a result of normal metabolic processes. However, when the production of these free radicals such as hydroxyl, peroxyl and superoxide radicals exceeds the threshold limit due to some physiological factors it leads to generation of oxidative stress (Benharlal and Arumughan 2007). This oxidative stress can cause severe oxidative damage to the biological macromolecules such as proteins, lipids, carbohydrates, nucleic acids directly or indirectly. Besides, oxidative stress can trigger several cellular processes like vasodilation, signal transduction, cell differentiation, development and a number of degenerative diseases such as diabetes, atherosclerosis, inflammatory diseases, cancer etc. (Islam et al. 2013).

Though the generation of excessive oxidative stress induces a remarkable impact on cellular and physiological processes of living systems; living cells constitute a unique and powerful radical scavenging machinery to counteract cellular injury. However, under the influence of ageing and other physiological factors these endogenous systems become inefficient and require exogenous antioxidant supplementation. In this context, there is an increasing attention towards the use of natural antioxidants such as ascorbic acid, phenolic compounds from plant origin in

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attenuating oxidative stress by maintaining the redox system of the living system (Mohamed et al. 2013).

*Hibiscus sabdariffa* L., (Malvaceae), commonly known as "roselle", is an important medicinal plant native to India and Malaysia. *H. sabdariffa* is rich in protocatechuic acid and anthocyanin which enables the plant extracts in effective treatment of cancer, hypertension, inflammation, mutagenecity, leukaemia and gastrointestinal disorders (Mohd-Esa et al. 2010; Yin et al. 2011). *H. sabdariffa* extracts from calyces have been reported for significant antioxidant properties due to presence of bioactive compounds such as delphinidin-3-O-glucoside, delphinidin-3-O-sambubioside, cyanidin-3-O-sambubioside, polyphenolic compounds and organic acids (Formagio et al. 2015; Tahir et al. 2016).

*Croton caudatus* Geisel. is an extensive plant of family Euphorbiaceae with potent therapeutic uses in convulsions, malaria, gastrointestinal disorders, rheumatic arthritis, liver disorders, cancer etc. (Nath et al. 2013; Rosangkima and Jagetia 2015). *C. caudatus* stem extract contains a majority of flavonoids such as sinensetin, kaemferol, nobiletin, crotoncaudatin, tangeretin etc. which aid in free radical scavenging activity (Zou et al. 2010).

In the present study, the antioxidant potential of *H. sabdariffa* and *C. caudatus* leaf extract was evaluated in *Saccharomyces cerevisiae* model system using the antioxidant mutants, *sod1Δ* and *tsa1Δ*. Hence, the present study aims to exploit the mechanism of radical scavenging in the sub-cellular system of yeast mutants, deficient in endogenous antioxidant mechanism. As Superoxide dismutase (SOD) and thioredoxin peroxidase (TSA) are mainly responsible for determining the oxidative stress response in *S. cerevisiae*, *sod1Δ* and *tsa1Δ* are frequently exploited for evaluating the scavenging efficacy of antioxidants in deficient endogenous antioxidant system (Kerdsomboon et al. 2015). Therefore, in the present study, antioxidant potential of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* were evaluated in *S. cerevisiae* model system.

## Materials and methods

### Chemicals and reagents

The chemicals used in the present study are ascorbic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), sulphuric acid ($H_2SO_4$), ammonium molybdate ($[NH_4]_6Mo_7O_2{\cdot}4H_2O$), 2-deoxyribose, ethylene diamine tetraacetic acid (EDTA), thiobarbituric acid (TBA), ferrous sulphate ($FeSO_4$), hydrogen peroxide ($H_2O_2$), sodium hydroxide (NaOH) and dichloro-fluorescin diacetate (DCF-DA). All the chemicals were purchased from Himedia Labratories, India.

### Collection of plant samples

The leaf samples of plants *H. sabdariffa* and *C. caudatus* were collected from Manipur, India. The leaf samples were shade dried and grounded to coarse powder form before extraction.

### Extract preparation

For sample preparation, 50 g of samples were extracted twice in 200 mL of ethanol and kept for 2–3 days in continuous shaking condition (150 rpm). The obtained extracts were filtered and the filtrates were concentrated using rotary evaporator at 40 °C (Saeed et al. 2012).

### Determination of in vitro antioxidant activity

#### *DPPH radical scavenging assay*

The free radical scavenging activity of ethanolic extract of *H. sabdariffa* and *C. caudatus* was determined in vitro by DPPH radical scavenging assay according to the method described by Eshwarappa et al. 2014 with slight modification. Briefly, 0.2 mM DPPH (in methanol) was mixed with different concentration of the sample (100–500 µg/mL) and incubated in the dark for 30 min at room temperature. The absorbance was taken at 517 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\begin{aligned}&\text{Scavenging effect } (\%)\\&\quad = [(\text{control absorbance} - \text{sample absorbance})\\&\qquad /\text{control absorbance}] \times 100.\end{aligned}$$

#### *Reducing power*

The reducing power assay was based on the reduction of ferric to ferrous form indicated by the formation of Prussian blue complex at 700 nm. Briefly, different concentrations of the sample were mixed with phosphate buffer (0.2 M, pH 6.6) and 1% $K_3Fe(CN)_6$. The reaction mixture was incubated at 50 °C for 20 min followed by addition of 10% TCA. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with deionized water and 0.1% (w/v) of freshly prepared $FeCl_3$. After 10 min of reaction, the absorbance was measured at 700 nm (Aqil et al. 2012).

#### *Hydroxyl radical scavenging assay*

The effect of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* on hydroxyl radicals was determined by using the

deoxyribose method described by Tounkara et al. 2014 with slight modifications (Tounkara et al. 2014). The reaction mixture contained 0.2 M sodium phosphate buffer (pH 7.0), 10 mM 2-deoxyribose, 10 mM $FeSO_4$-EDTA, 10 mM $H_2O_2$ and different concentration of sample (100–500 µg/mL). The reaction was started by the addition of $H_2O_2$ and incubated at 37 °C for 4 h followed by the addition of 2.8% TCA and 1% TBA in 50 mM NaOH. The resulting solution was boiled in a water bath for 10 min and then cooled to room temperature. The absorbance of the solution was measured at 532 nm. The ability to scavenge the hydroxyl radical was calculated using the following equation:

$$\% \text{ of inhibition} = [(\text{control absorbance} - \text{sample absorbance}) / \text{control absorbance}] \times 100.$$

*Total antioxidant assay*

The total antioxidant activity of ethanolic extract of *H. sabdariffa* and *C. caudatus* was determined by phosphomolybdate method. Briefly, different concentration of sample (100–500 µg/ml) solution was mixed with reagent solution (comprising of 0.6 M $H_2SO_4$, 28 mM sodium phosphate and 4 mM ($[NH_4]_6Mo_7O_2{\cdot}4H_2O$)). The reaction mixture was incubated in a water bath at 95 °C for 90 min followed by cooling at room temperature. The absorbance of the mixture was measured at 695 nm. The total antioxidant activity of the ethanolic fractions of the plants was expressed as ascorbic acid equivalents (Kalaivani et al. 2011).

*GC–MS analysis*

The presence of important secondary metabolites in ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* was determined by GC–MS analysis. The spectrums of the components were compared with the GC–MS NIST (2008) library (de Almeida et al. 2013).

**Determination of in vivo antioxidant activity**

*Yeast strain, media and growth condition*

In the present study, wild type strain BY4741 and their isogenic deletion strains *sod1Δ* and *tsa1Δ* of *S. cerevisiae* were used to validate the antioxidant efficacy of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus*. Yeast cells were obtained after the middle of first exponential phase ($OD_{600} = 0.5$) in liquid YPD medium (1% yeast extract, 2% peptone and 2% glucose) using an orbital shaker at 160 rpm and 30 °C (Frassinetti et al. 2012).

*$H_2O_2$ sensitivity assay*

To evaluate the sensitivity against oxidative agents ($H_2O_2$), overnight cultures of yeast strains were used. Briefly, 20 µL of exponential phase of the *S. cerevisiae* cultures were taken and serially diluted in a 96-well plate and spotted onto different concentration of $H_2O_2$ (1, 1.5, 2, 2.5, 3 and 4 mM) treated YPD agar (YPDA) plates and incubated at 30 °C for 24–48 h to assess the sensitivity of the yeast mutants to a varied degree of stressing agent (Wu et al. 2011).

*Spot assay*

Wild type *S. cerevisiae* and their mutant strains were used to assess the antioxidant efficacy of ethanolic leaf extracts of *H. sabdariffa* and *C. caudatus*. Briefly, overnight *S. cerevisiae* cells ($OD_{600} = 0.5$) with and without exposure to ethanolic plant extracts (500 µg/mL) and oxidative agent (2.5 mM) were incubated for 1 h each in YPD medium at 160 rpm and 30 °C. After incubation, subsequent dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$) were made for all the strains in a 96 well-plate. The cells were then spotted onto YPDA plates and incubated at 30 °C for 24 h (Golla and Raj Bhimathati 2014).

*ROS detection assay*

DCF-DA method was performed to detect the level of cellular ROS and their subsequent removal by ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* as described by Azad et al. 2014 with slight modifications (Azad et al. 2014). Briefly, cells were grown in YPD medium until $A_{600}$ reached 0.5. The cultures were treated with different concentration of ethanolic leaf extracts (100–500 µg/mL) and incubated at 30 °C for 1 h $H_2O_2$ (1 mM) was added to the reaction mixture and incubated for 30 min followed by centrifugation (6000 rpm, 10 min) to collect the cells. The cells were then washed thrice with 1X phosphate-buffered saline (PBS). Cells were resuspended in PBS with 0.8 µL of 20 µM 2′, 7′-dichlorofluorescein diacetate (DCF-DA) and incubated at 30 °C for 1 h in dark. The fluorescent dye reacts specifically with $H_2O_2$ to give a highly fluorescent DCF. Cells were collected, washed three times with PBS, of which 10 µL was mounted onto slides and examined under fluorescent microscope with an excitation and emission wavelength of 485 and 529 nm respectively. The remaining cells were transferred into 96-well plate and fluorescent intensity of the samples was measured using

plate reader. A blank was prepared consisting of PBS without any sample.

### Statistical analysis

All the experiments were conducted in triplicate. All the values were represented as mean ± standard deviation. All the in vitro antioxidant results and datas obtained from the fluorescent intensity studies were subjected for Tukey–Kramer multiple comparison test (Q-test) followed by one-way ANOVA to analyze whether there exist significant difference in the antioxidant activities shown by the test samples as compared to the control at varied level of significance. For statistical analyses, $p$ values <0.05 were considered significant (Huang et al. 2015).

## Results and discussion

### Determination of in vitro antioxidant activity

#### *DPPH radical scavenging assay*

The ability of ethanolic leaf extracts to scavenge free radicals was determined by DPPH assay. DPPH free radical scavenging activity exhibited scavenging potential of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* in a concentration dependent manner. From the results, it was observed that *H. sabdariffa* showed a scavenging potential of 65.19 ± 1.63% at 500 µg/mL with an $IC_{50}$ value of 184.88 µg/mL which was comparatively higher than the previous report (Tahir et al. 2016). Meanwhile, *C. caudatus* showed 57.86 ± 1.69% of free radical scavenging (Table 1). The $IC_{50}$ value for *C. caudatus* was observed to be 305.39 µg/mL is significantly higher than the earlier report with an $IC_{50}$ of 396.20 µg/mL (Qiasar et al. 2013).

#### *Reducing power*

In the present study, an increase in the absorbance was observed with a concomitant increase in the concentration of ethanolic leaf extracts with a characteristic change in colour from yellow to green in colour which eventually enhances the reducing power. In the present study, out of the 2 plants studied, *H. sabdariffa* showed higher reducing power as compared to *C. caudatus* as evident from their absorbance of 0.43 ± 0.01 and 0.375 ± 0.01 respectively at a concentration of 500 µg/mL (Table 2). The result of present study was in accordance with the previous report (Tounkara et al. 2014).

#### *Hydroxyl radical scavenging*

From the $OH^{\cdot}$ radical scavenging results, it was observed that the ethanolic leaf extract scavenged highly reactive $OH^{\cdot}$ radical in a concentration dependent manner. In the present study, *H. sabdariffa* showed relatively higher $OH^{\cdot}$ radical scavenging (65.55 ± 2.45%) with an $IC_{50}$ of 281.42 µg/mL at a concentration of 500 µg/mL This result was comparatively higher than the previous report with a scavenging percentage of 62.30% at a concentration of 10 mg/mL (Tounkara et al. 2014). Meanwhile, *C. caudatus* extract exhibited 59.99 ± 1.52% of $OH^{\cdot}$ radical scavenging activity with an $IC_{50}$ of 335.07 µg/mL (Table 1). The potential of scavenging highly reactive $OH^{\cdot}$ radicals by the ethanolic extract of *H. sabdariffa* and *C. caudatus* suggested its efficacy in minimizing the effects of lipid peroxidations in biological systems.

**Table 1** DPPH free radical scavenging and hydroxyl radical scavenging potential of ethanolic leaf extract of *Croton caudatus* and *Hibiscus sabdariffa* at 500 µg/mL

| Ethanolic leaf extract and positive control | DPPH radical scavenging activity | | OH radical scavenging activity | |
|---|---|---|---|---|
| | Percentage of DPPH activity (%) | $IC_{50}$ (µg/mL) | Percentage of $OH^{\cdot}$ radical scavenging (%) | $IC_{50}$ (µg/mL) |
| *C. caudatus* | 57.86 ± 1.69* | 305.39 | 59.99 ± 1.52*** | 335.07 |
| *H. sabdariffa* | 65.19 ± 1.63* | 184.88 | 65.55 ± 2.45 | 281.42 |
| Ascorbic acid | 74.68 ± 3.19 | 2.83 | 67.37 ± 2.88 | 3.83 |

Radical scavenging percentage values were represented as Mean ± standard deviation (SD) of 3 replicates (n = 3). Ascorbic acid was used as positive control

All the values are expressed as Mean ± SD (n = 3)

* Values are significantly different from positive control (ascorbic acid) at $p < 0.05$

** Values are significantly different from positive control (ascorbic acid) at $p < 0.01$

*** Values are significantly different from positive control (ascorbic acid) at $p < 0.001$

**Table 2** Reducing Power and total antioxidant activity of ethanolic extract of *Croton caudatus* and *Hibiscus sabdariffa* at 500 µg/mL

| Ethanolic leaf extract and positive control | Reducing power (absorbance at 700 nm) | Total antioxidant activity (Ascorbic acid equivalents in µg/mL) |
|---|---|---|
| *C. caudatus* | 0.387 ± 0.02*** | 77.07 ± 4.16* |
| *H. sabdariffa* | 0.439 ± 0.016* | 96.72 ± 4.88 |
| Ascorbic acid | 0.479 ± 0.026 | |

All the values were represented as Mean ± standard deviation (SD) of 3 replicates (n = 3). Ascorbic acid was used as positive control

All the values are expressed as Mean ± SD (n = 3)

* Values are significantly different from positive control (ascorbic acid) at $p < 0.05$

** Values are significantly different from positive control (ascorbic acid) at $p < 0.01$

*** Values are significantly different from positive control (ascorbic acid) at $p < 0.001$

*Total antioxidant activity*

The total antioxidant activity of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* revealed that with an increase in concentration there is an increase in absorbance which corresponds to the enhanced total antioxidant activity. The ethanolic extract of *H. sabdariffa* and *C. caudatus* showed an ascorbic acid equivalent of 96.72 ± 4.88 and 77.07 ± 4.16 µg/mL respectively (Table 2). This result coincide with the previous report where the ethanolic extract showed the most prominent antioxidant activity as compared to other extraction medium (Kalavani et al. 2011).

*GC–MS analysis*

GC–MS analysis of ethanolic leaf extract of *H. sabdariffa* showed the presence of phytoconstituents like phytol, alpha-tocopherol, methyl linolenate, ethyl palmitate and ethyl linolenate in substantial quantity. Meanwhile, ethanolic leaf extract of *C. caudatus* showed the presence of phytol and hexamethyl cyclotrisiloxane in significant quantity in the GC–MS analysis (Table 3).

The presence of natural antioxidants such as phytol and alpha tocopherol as phytoconstituents in the ethanolic extracts of *H. sabdariffa* leaves suggested the enhancement in the antioxidant activity in a multivariate manner both in vitro as well as in cellular stress response system in *S. cerevisiae* (Santos et al. 2013; Silva et al. 2013; Pejin et al. 2014; Vargas et al. 2014; Zhang et al. 2016; Shah et al. 2016). In addition to these phytoconstituents, the prevalence of fatty acids and fatty acid esters also aid to a significant increase in the antioxidant potential of *H. sabdariffa* in *S. cerevisiae* system which is reported for the first time (Sudha et al. 2013; Sharma et al. 2015). In the present study, *C. caudatus* leaf extract also exhibited significant antioxidant activity due to presence of substantial amount of phytol and hexamethyl cyclotrisiloxane as evident from the GC–MS analysis (Shibula and Velavan 2015; Falowo et al. 2016).

## In vivo antioxidant activity

*$H_2O_2$ sensitivity*

$H_2O_2$ sensitivity test revealed that *S. cerevisiae* isogenic deletion mutants (*sod1Δ* and *tsa1Δ*) donot showed any sign of sensitivity until the concentration of $H_2O_2$ reached 2.5 mM. However, with an increase in concentration, both mutant strains invariably showed varied degree of sensitivity. Hence, 2.5 mM of $H_2O_2$ was used for subsequent in vivo studies.

*Spot assay*

A significant difference in the growth pattern was observed in the stressed yeast cells in presence or absence of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus*. This suggested the efficacy of the plant extract in ameliorating the stress generated by $H_2O_2$ in antioxidant deficient *sod1Δ* and *tsa1Δ* mutants. In the present study, out of the 2 test plants *H. sabdariffa* showed comparatively higher potential to scavenge the stress generated by $H_2O_2$ (Fig. 1). The spot assay suggested the efficacy of *H. sabdariffa* and *C. caudatus* leaf extract by decreasing the cytotoxicity generated by $H_2O_2$ (Slatnar et al. 2012).

*ROS detection*

The ability of ethanolic extract of *H. sabdariffa* and *C. caudatus* to protect the yeast cells from the damaging effect of oxidative stress was examined by DCF-DA assay by measuring the level of intracellular $H_2O_2$. The evaluation of intracellular ROS level acts as a prime marker to determine the level of oxidative damage in yeast cells in absence/presence of plant extracts (Frassinetti et al. 2012). The results showed that the level of fluorescence was comparatively higher in stressed *S. cerevisiae* mutant strains in absence of any plant extracts (negative control), as the fluorescent stain specifically fluoresce more with

**Table 3** List of phytochemicals present in the ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* from the GC–MS analysis

| Sl no. | Compound name | Common name | Retention time (RT) (min) | Biological activities | References |
|---|---|---|---|---|---|
| Ethanolic leaf extract of *Hibiscus sabdariffa* | | | | | |
| 1. | Hexadecanoic acid ethyl ester | Ethyl palmitate | 18.13 | Antioxidant | Sudha et al. 2013 |
| 2. | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | Phytol | 16.759 | Antiradical, antimicrobial, antioxidant, antityrosinase, antinociceptive activity, anti-inflammatory activity | Santos et al. 2013; Silva et al. 2013; Pejin et al. 2014; Zhang et al. 2016 |
| 3. | Alpha tocopherol | Vitamin E | 27.173 | Radical scavenging activity, antioxidant activity | Vargas et al. 2014; Shah et al. 2016 |
| 4. | 9,15-octadecadienoic acid, methyl ester | Methyl linolenate | 19.66 | Antioxidant activity | Sharma et al. 2015 |
| 5. | 9,12,15-octadecatrienoic acid, ethyl ester | Ethyl linolenate | 19.75 | Antioxidant activity | Sharma et al. 2015 |
| Ethanolic leaf extract of *Croton caudatus* | | | | | |
| 1. | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | Phytol | 17.02 | Antioxidant | Pejin et al. 2014; Zhang et al. 2016; Shibula and Velavan 2015 |
| 2. | Cyclotrisiloxane, hexamethyl | Hexamethyl Cyclotrisiloxane | 29.32 | Antioxidant | Falowo et al. 2016 |

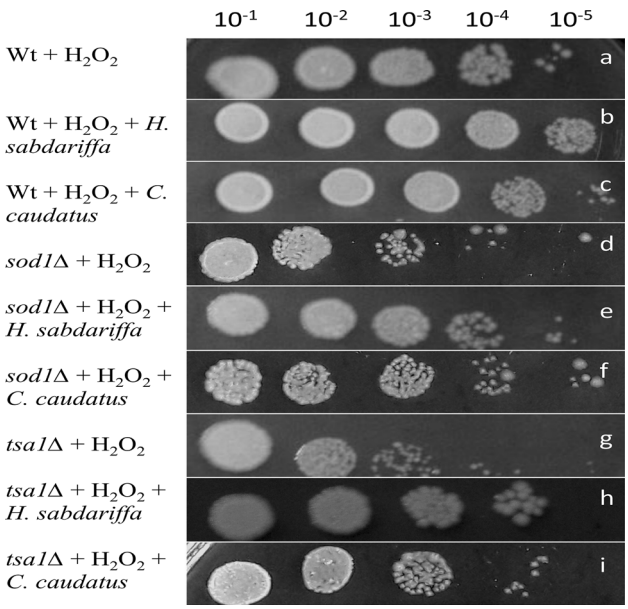


**Fig. 1** Effect of ethanolic leaf extracts of *Hibiscus sabdariffa* and *Croton caudatus* on the growth of yeast cells (wild type and mutants) by spot assay. **a** Wt yeast cells treated with $H_2O_2$, negative control; **b** Wt yeast cells treated with $H_2O_2$ and ethanolic leaf extract of *H. sabdariffa*; **c** Wt yeast cells treated with $H_2O_2$ and ethanolic leaf extract of *C. caudatus*; **d** *sod1Δ* yeast cells treated with $H_2O_2$, negative control; **e** *sod1Δ* yeast cells treated with $H_2O_2$ and ethanolic leaf extract of *H. sabdariffa*; **f** *sod1Δ* yeast cells treated with $H_2O_2$ and ethanolic leaf extract of *C. caudatus*; **g** *tsa1Δ* yeast cells treated with $H_2O_2$, negative control; **h** *tsa1Δ* yeast cells treated with $H_2O_2$and ethanolic leaf extract of *H. sabdariffa*; **i** *tsa1Δ* yeast cells treated with $H_2O_2$ and ethanolic leaf extract of *C. caudatus*

concomitant higher level of subcellular $H_2O_2$. However, on treatment with *H. sabdariffa* and *C. caudatus* leaf extract, the fluorescence significantly decreased as evident from fluorescent intensity studies that can be correlated with concomitant decrease in the intracellular ROS level in *S. cerevisiae* mutants (Figs. 2, 3).

*H. sabdariffa* showed the highest scavenging potential with a percentage decrease of 59.13, 61.88 and 75.10% in the fluorescence intensity in wild type, *sod1Δ* and *tsa1Δ* mutant strains respectively as compared to the negative control. Meanwhile, ethanolic leaf extract of *C. caudatus* exhibited 53.03, 56.01 and 59.65% decrease in fluorescence intensity in wild type, *sod1Δ* and *tsa1Δ* mutant strains respectively as compared to the negative control. From the results, it was observed that both *H. sabdariffa* and *C. caudatus* are highly reactive towards *tsa1Δ* mutant as evident from higher percentage decrease in fluorescence intensityas compared to the negative control. The reason behind the decrease in fluorescence intensity in the plant extract treated yeast cells might be the low level of intracellular ROS which is insufficient to oxidize DCF-DA to highly fluorescent DCF (Wu et al. 2009).

Due to excessive production of ROS, the endogenous antioxidant system may fail to scavenge the free radicals in an effective manner. In this context, there is a growing demand for supplementing natural antioxidants exogenously to maintain a proper balance in the system as the application of synthetic antioxidants possess severe

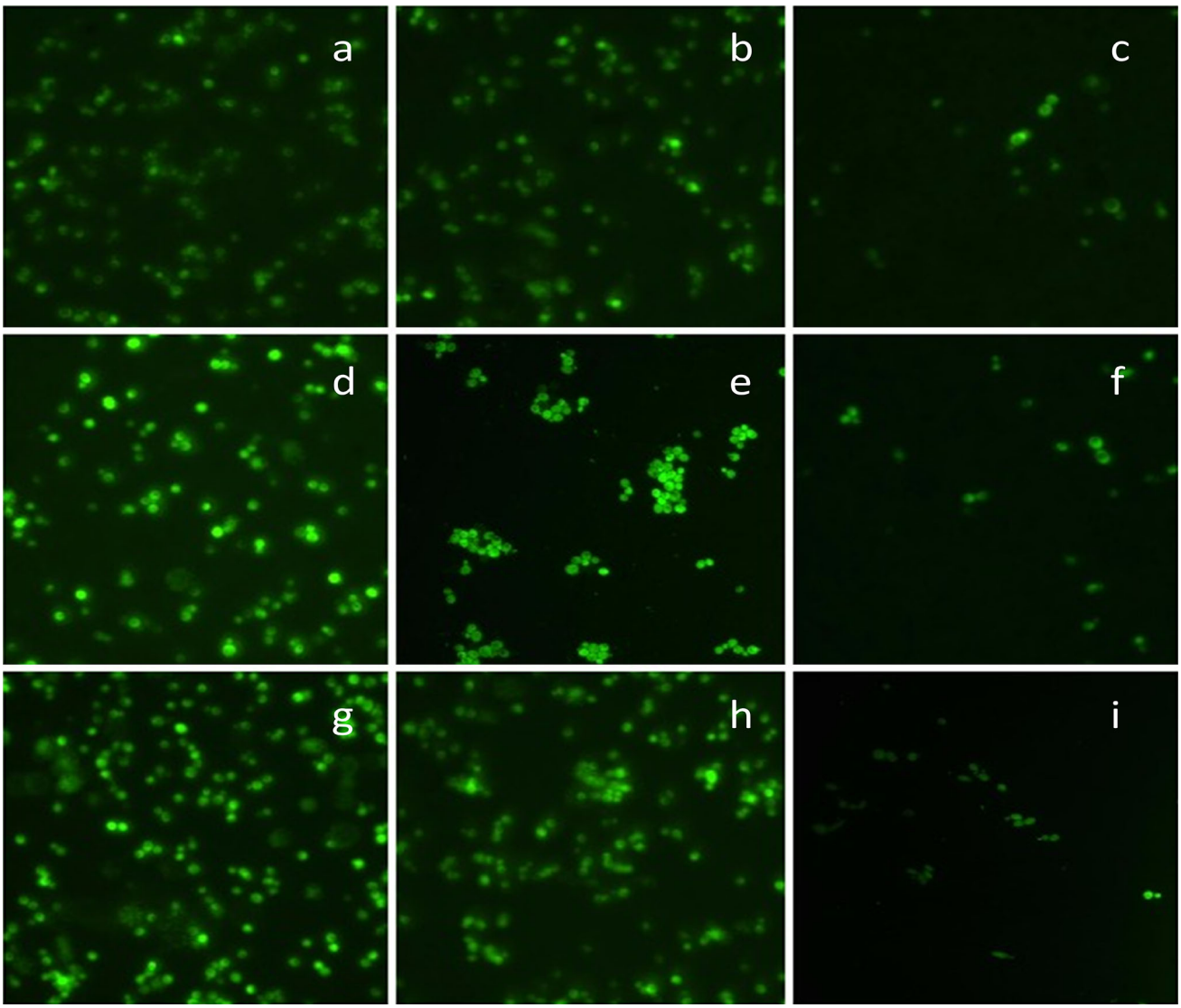


**Fig. 2** Determination of intracellular reactive oxygen species (ROS) in the *S. cerevisiae* cells (both Wt and mutants) by fluorescent microscopic studies using DCF-DA assay under the influence of ethanolic extract of *Hibiscus sabdariffa* and *Croton caudatus*. **a** Wt yeast cells treated only with $H_2O_2$, negative control; **b** Wt yeast cells treated with $H_2O_2$ and *C. caudatus*; **c** Wt yeast cells treated with $H_2O_2$ and *H. sabdariffa*; **d** *sod1Δ* yeast cells treated with $H_2O_2$, negative control; **e** *sod1Δ* yeast cells treated with $H_2O_2$ and *C. caudatus*; **f** *sod1Δ* yeast cells treated with $H_2O_2$ and *H. sabdariffa*; **g** *tsa1Δ* yeast cells treated only with $H_2O_2$, negative control; **h** *tsa1Δ* yeast cells treated with $H_2O_2$ and *C. caudatus*; **i** *tsa1Δ* yeast cells treated with $H_2O_2$ and *H. sabdariffa*

carcinogenic properties and other health ailments (Wang et al. 2014). Plants and plant derived products are the richest source of phytoconstituents and can be utilized for attenuating the oxidative stress and their side effects (Aderogba et al. 2011). In that context, in the present study, an attempt was made to evaluate the scavenging potential of ethanolic leaf extract of *H. sabdariffa* and *C. caudatus* in attenuating the level of ROS generated in the stress response system in *S. cerevisiae* wild type and their mutants as model system for the 1st time.

From the in vitro and in vivo antioxidant studies of *H. sabdariffa* and *C. caudatus* leaf extract unwrapped the efficacy of these plant extracts as an efficient antioxidant in *S. cerevisiae* model system which was reported for the first time. This result suggested the use of these plants as remarkable alternative to synthetic antioxidants and thus established their use in ethnopharmacological applications.

## Conclusion

In the present study, both *H. sabdariffa* and *C. caudatus* leaf extract showed significant antioxidative potential in vitro as well as in the *S. cerevisiae* model system specifically

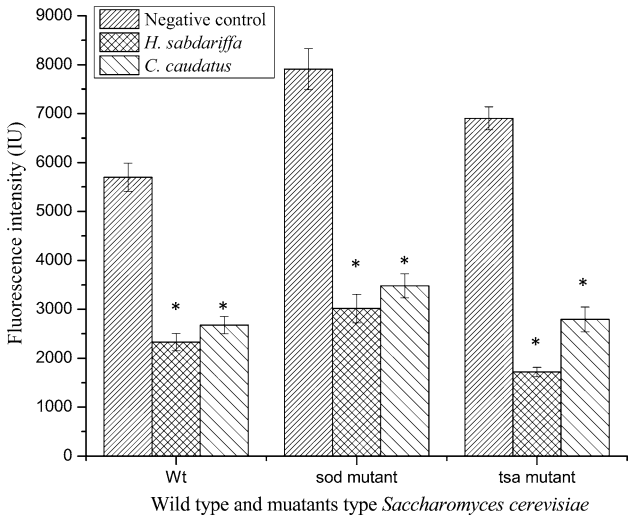


**Fig. 3** Effect of ethanolic leaf extract of *Hibiscus sabdariffa* and *Croton caudatus* on the intracellular ROS levels estimated by the intensity of fluorescence. All the values were represented as mean ± SD (n = 3). *** Values are significantly different from positive control (ascorbic acid) at $p < 0.001$, ** values are significantly different from positive control (ascorbic acid) at $p < 0.01$, * values are significantly different from positive control (ascorbic acid) at $p < 0.05$

targeting the antioxidant deficient mutant yeast cells, suggesting their multitudinal application in radical scavenging activity and widespread pharmacological potential.

**Compliance with ethical standards**

**Conflict of interest** The authors are declaring no conflict of interests in this work.

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