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Research Article

COMPARATIVE ANTIOXIDANT ACTIVITY OF THE SYNTHESIZED (*E*)-CHALCONES

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ABSTRACT
Depletion of dietary antioxidants has been related to rising of oxidative stress that causes chronic and degenerative diseases such as cancers, Alzheimer and aging. Therefore, finding of a readily available antioxidant is essential to offer potential chemotherapeutics. In this study, (*E*)-1,3-diphenylprop-2-en-1-one (**1**), (*E*)-1-(3-nitrophenyl)-3-phenylprop-2-en-1-one (**2**), (*E*)-3-(furan-2-yl)-1-(3-nitropheyl)prop-2-en-1-one (**3**), (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (**4**), (*E*)-3-(furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5**), (*E*)-1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**6**) and (*E*)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**7**), that readily obtained through the Crossed-Aldol condensation between arylmethyl ketones and aromatic aldehydes, were evaluated for the antioxidant activity by using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Nitric oxide (NO) assays. Among the chalcones investigated, compound **7** has displayed antioxidant activity in the ABTS assay with IC_{50} value of 464 μM (calcd. 124 μg/mL) indicating a *para*-dimethylamino substitution in the B ring of chalcone enhances reduction of cationic free radical $ABTS^{\bullet+}$.

Keywords: Anticancer, 1,3-Diaryl-2-propene-1-one, Crossed-Aldol condensation, Flavonoid, IC_{50}, Oxidative stress

INTRODUCTION
Pro-oxidant-antioxidant imbalance in the body is the central cause of oxidative stress. The homeostatic concentration of free radicals formed during metabolisms is equilibrated by antioxidant defences reducing oxidative stress. Increased generation of free radicals and their poor scavenging in the body by depletion of the dietary antioxidants can cause extensive cellular damage that eventually leads to several health problems including cancers, ulcers, arthritis and aging. In the recent past, free radical treatment has upsurged interest to cure oxidative stress-related diseases (Thirunavukkarasu *et al.*, 2019).

Chalcones (1,3-diaryl-2-propene-1-ones) (Fig. 1) are precursors of flavonoids and possess various biological activities including antioxidant property (Singh *et al.*, 2014; Thapa *et al.*, 2016; Gaonkar & Vignesh, 2017; Jasim *et al.*, 2021). Antioxidant activity is primarily considered to be associated with hydroxyl and phenyl substituents (Anto *et al.*, 1995).

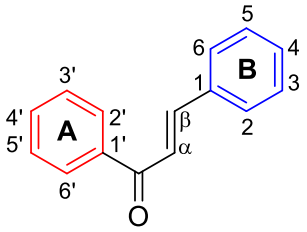


Figure 1. General structure of chalcone.

Several bioassay techniques have been developed to measure antioxidant activity of biological and chemical samples (Sadeer *et al.*, 2020). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is based on either single electron transfer (SET) mechanism, hydrogen atom transfer (HAT) mechanism or both (Fig. 1a) (Huang *et al.*, 2005; Rubio *et al.*, 2016). The 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay relies on SET mechanism where a radical cation $ABTS^{\bullet+}$ is generated by treating ABTS with $K_2S_2O_8$, and subsequently reduced by the antioxidant (Fig. 1b) (Miller *et al.*, 1993). On the other hand, the Nitric oxide (NO) assay depends on the ability of the antioxidant to block the formation of nitric oxide radical ($NO^{\bullet}$) (Griess, 1864). $NO^{\bullet}$ reacts with atmospheric O_2 to produce nitrite (NO_2^-) that quantifies by Griess reagent, but in the presence of an antioxidant, nitrite cannot be produced (Fig. 1c) (Grossi & D'Angelo, 2005; Tsikas, 2007; Hetrick & Schoenfisch, 2009). Hence, the results of these assays in the evaluation of antioxidant capacity of a sample may alter due to association of different mechanistic pathways.

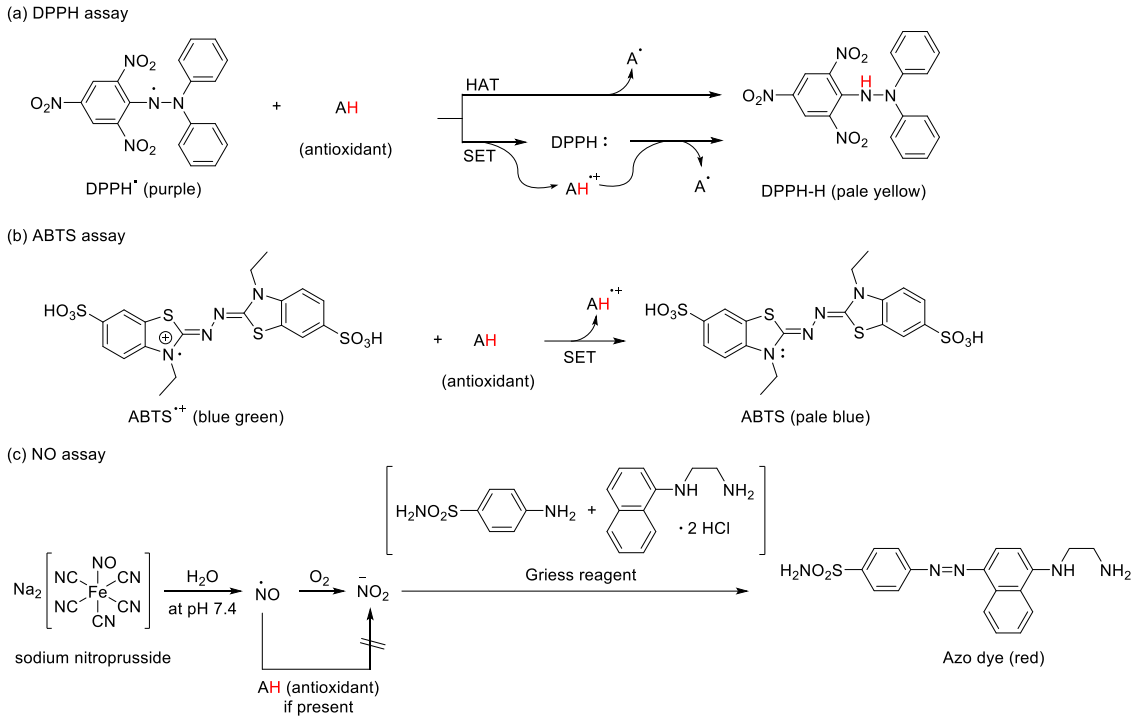


Figure 2. Reaction mechanisms involved in the DPPH, ABTS and NO assays.

In continuation of our work for the evaluation antioxidant activity of different groups of molecules (Thapa *et al.*, 2016; Bajracharya *et al.*, 2020; Gupta *et al.*, 2021), herein, we compare the antioxidant activity of some simple (*E*)-chalcones by using three different assays *viz*. DPPH, ABTS and NO assays.

MATERIALS AND METHODS

General information

Chemicals were purchased from Fischer, Qualigens, Aldrich, Merck and Loba. Gallic acid was procured from Sisco Research Laboratories Pvt. Ltd. Melting point (M.p.) determined using a Thiel's tube was uncorrected. UV spectra were recorded on Cary 60 UV-Vis (Agilent). IR spectra were recorded with IRTracer 100 (Shimadzu). NMR spectra were recorded on Bruker Avance III 300 and Bruker Avance Neo 400 spectrometers. BioTek Epoch 2 (Agilent) was used for spectrophotometry. Reaction monitoring was performed by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} plates (Sigma-Aldrich, Canada).

Synthesis of (*E*)-chalcones

(E)-1,3-Diphenylprop-2-en-1-one (1)

In a stirring solution of NaOH (1.28 g), distilled water (12 mL) and EtOH (7 mL) at 0 °C, acetophenone (2.92 mL, 25 mmol) and benzaldehyde (2.54 mL, 25 mmol) were added slowly. The mixture was stirred at room temperature for 3 hr, and then was kept in a refrigerator for overnight. Thus formed precipitate of compound **1** was collected and washed with cold water (Kohler & Chadwell, 1922; Thapa *et al.*, 2016). Light yellow solid. Yield 4.273 g, 82.1%. M.p. 54 °C. R_f = 0.85 (silica gel, hexane/EtOAc, 7:3). UV (MeOH) λ nm: 309, 227. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.03 (d, *J* = 6 Hz, 2H), 7.82 (d, *J* = 15 Hz, 1H, β-H), 7.67-7.64 (m, 2H), 7.59-7.49 (m, 3H), 7.53 (d, *J* = 15 Hz, α-H), 7.43-7.41 (m, 3H) (Zhao & Song, 2016). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 190.68, 144.96, 138.38, 135.05, 132.90, 130.67, 129.10, 128.76, 128.64, 128.58, 122.28.

(E)-1-(3-Nitrophenyl)-3-phenylprop-2-en-1-one (2)

In a stirring solution of NaOH (0.80 g), distilled water (6 mL) and EtOH (6 mL) at 0 °C, *m*-nitroacetophenone (1.980 g, 12 mmol) and benzaldehyde (1.22 mL, 12 mmol) were added slowly. The mixture was stirred at room temperature for 3 hr, and then was kept in a refrigerator for overnight. Thus, formed precipitate of compound **2** was collected and washed with cold water. The product was further purified by column chromatography (silica gel, 100-200 mesh; hexane/EtOAc, 9:1) (Thapa *et al.*, 2016). Cream coloured solid. Yield 1.220 g, 40%. R_f = 0.75 (silica gel, hexane/EtOAc, 7:3). UV (MeOH) λ nm: 315, 238. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.83 (t, *J* = 3 Hz, 1H), 8.46-8.45 (m, 1H), 8.35 (dt, *J* = 6, 3 Hz, 1H), 7.89 (d, *J* = 15 Hz, 1H, β-H), 7.73 (d, *J* = 9 Hz, 1H), 7.69-7.66 (m, 2H), 7.53 (d, *J* = 15 Hz, 1H, α-H), 7.46-7.44 (m, 3H) (Zhao & Song, 2016). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 188.13, 148.58, 146.90, 139.66, 134.47, 134.21, 131.34, 130.04, 129.24, 128.87, 127.18, 123.40, 120.82.

(E)-3-(Furan-2-yl)-1-(3-nitrophenyl)prop-2-en-1-one (3)

In a stirring solution of NaOH (0.40 g), distilled water (3 mL) and EtOH (3 mL) at 0 °C, *m*-nitroacetophenone (0.990 g, 6 mmol) and furfuraldehyde (0.5 mL, 6 mmol) were added slowly. The mixture was stirred at room temperature for 3 hr, and then was kept in a refrigerator for overnight. Compound **3** was extracted with EtOAc

(30 mL × 3) followed by washing with brine, drying over Na_2SO_4, filtration, concentration and purification by column chromatography (silica gel, 100-200 mesh; hexane/EtOAc, 9:1) (Thapa *et al.*, 2016). Yellow-cream coloured solid. Yield 0.729 g, 43%. R_f = 0.69 (silica gel, hexane/EtOAc, 8:2). UV (MeOH) λ nm: 347, 246. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.84 (t, *J* = 3 Hz, 1H), 8.42 (q, *J* = 9 Hz, 1H), 8.35 (td, *J* = 3, 6 Hz, 1H), 7.73-7.68 (m, 1H), 7.66 (d, *J* = 15 Hz, 1H, β-H), 7.57 (s, 1H), 7.44 (d, *J* = 15 Hz, 1H, α-H), 6.80 (d, *J* = 3 Hz, 1H), 6.55 (d, *J* = 9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 187.43, 151.44, 148.60, 145.76, 139.64, 134.12, 132.28, 130.00, 127.13, 123.34, 117.97, 117.73, 113.12.

(E)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (4)

In a stirring solution of NaOH (1.60 g), distilled water (6 mL) and EtOH (3 mL) at 0 °C, 2-hydroxyacetophenone (1.44 mL, 12 mmol) and benzaldehyde (1.22 mL, 12 mmol) were added slowly. The mixture was stirred at room temperature for 30 hr, and then quenched by 10% HCl (30 mL). Compound **4** was extracted with EtOAc (30 mL × 3), washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by recrystallization with MeOH (Thapa *et al.*, 2016). Yellow crystals. Yield 1.178 g, 43.8%. R_f = 0.62 (silica gel, hexane/EtOAc, 9:1). UV (MeOH) λ nm: 316, 227. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.93 (d, *J* = 15 Hz, 1H, β-H), 7.93 (d, *J* = 9 Hz, 1H), 7.68-7.66 (m, 2H), 7.67 (d, *J* = 15 Hz, 1H, α-H), 7.51 (t, *J* = 9 Hz, 1H), 7.45-7.44 (m, 3H), 7.04 (d, *J* = 6 Hz, 1H), 6.95 (t, *J* = 6 Hz, 1H) (Mai *et al.*, 2013). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 193.88, 163.75, 145.60, 136.54, 134.75, 131.06, 129.80, 129.18, 128.80, 120.29, 120.17, 118.99, 118.79.

(E)-3-(Furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (5)

In a stirring solution of NaOH (1.60 g), distilled water (6 mL) and EtOH (30 mL) at 0 °C, 2-hydroxyacetophenone (1.44 mL, 12 mmol) and furfuraldehyde (0.99 mL, 12 mmol) were added slowly. The mixture was stirred at room temperature for 24 hr, and then quenched by 10% HCl (30 mL). Compound **5** was extracted with EtOAc (30 mL × 3), washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by recrystallization with MeOH (Thapa *et al.*, 2016). Yellow crystals. Yield 1.930 g, 85.1%. R_f = 0.64 (silica gel, hexane/EtOAc, 9:1). UV (MeOH) λ nm: 359, 247. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.92 (dd, *J* = 9, 3 Hz, 1H), 7.68 (d, *J* = 15 Hz, 1H, β-H), 7.55 (d, *J* = 15 Hz, 1H, α-H), 7.57-7.46 (m, 2H), 7.02 (d, *J* = 6 Hz, 1H), 6.97-6.91 (m, 1H), 6.77 (d, *J* = 3 Hz, 1H), 6.54 (d, *J* = 3 Hz, 1H) (Kumari *et al.*, 2017). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 193.47, 163.71, 151.70, 145.54, 136.44, 131.26, 129.78, 120.21, 118.98, 118.70, 117.81, 117.25, 113.03.

(E)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (6)

In a stirring solution of NaOH (1.60 g), distilled water (6 mL) and EtOH (30 mL) at 0 °C, 2-hydroxyacetophenone (1.44 mL, 12 mmol) and anisaldehyde (1.45 mL, 12 mmol) were added slowly. The mixture was stirred at room temperature for 48 hr, and then quenched by 10% HCl (30 mL). Compound **6** was extracted with EtOAc (30 mL × 3), washed with brine, dried over Na_2SO_4, filtered and concentrated (Thapa *et al.*, 2016). Yellow solid. Yield 2.136 g, 70%. R_f = 0.75 (silica gel, hexane/EtOAc, 7:3). UV (MeOH) nm: 365, 241. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.93-7.88 (m, 1H), 7.90 (d, *J* = 15 Hz, 1H, β-H), 7.62 (d, *J* = 9 Hz, 2H), 7.53 (d, *J* = 15 Hz, 1H, α-H), 7.47 (d, *J* = 9 Hz, 1H), 7.02 (d, *J* = 9 Hz, 1H), 6.97-6.91 (m, 3H), 3.86 (s, 3H) (Mai *et al.*, 2013). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 193.80, 163.70, 162.17, 145.48, 136.27, 130.68, 129.67, 127.50, 120.27, 118.88, 118.73, 117.75, 114.67, 55.59.

(E)-3-(4-(Dimethylamino)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (7)

In a stirring solution of 2'-hydroxyacetophenone (0.681 g, 5 mmol) and EtOH (25 mL), 4-dimethylaminobenzaldehyde (0.746 g, 5 mmol) was added slowly. To this was added 40% NaOH (20 mL), and stirring was continued at room temperature for 72 hr. The reaction mixture was diluted with cold water followed by neutralisation with 10% HCl and then kept overnight. Precipitate of compound **7** was filtered, washed with cold water-EtOH mixture, and purified by recrystallisation with EtOH (Singh *et al.*, 2016; Thapa *et al.*, 2022). Bright red crystals. Yield 0.695 g, 52%. M.p. 175 °C. R_f = 0.36 (silica gel, hexane/EtOAc, 1.7:0.3). UV (MeOH) λ nm: 274, 435. IR (KBr) $\bar{\upsilon}$ cm^{-1}: 3373 (OH), 1612 (C=O), 2970 (C-H), 1525 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 13.17 (s, OH), 7.92-7.88 (m, 2H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.46-7.42 (m, 2H), 6.99 (d, *J* = 8 Hz, α-H), 6.90 (t, *J* = 7.6 Hz, β-H), 6.68 (d, *J* = 8.8 Hz, 2H), 3.04 (s, 6H, NMe_2) (Singh *et al.*, 2016). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 193.72, 163.71, 152.48, 146.71, 135.86, 131.06, 129.57, 122.67, 120.61, 118.78, 118.70, 114.58, 112.10, 40.37.

Antioxidant assays

Gallic acid standard solutions of 5, 10, 25, 50 and 100 μM concentrations were prepared in methanol. Methanolic solutions of chalcones (**1**-**7**) were prepared in 10, 50, 100, 500, 1000 and 2000 μM concentrations. These solutions were stored in a refrigerator.

A. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH• solution (0.1 mM) was prepared by overnight stirring of DPPH (3.9 mg) in HPLC grade methanol (100 mL) (Brand-Williams *et al.*, 1995; Nemkul *et al.*, 2021; Nemkul *et al.*, 2022). The methanolic solutions of Gallic acid standard and chalcones (50 μL) were loaded in 96 well plates in triplicates, and then DPPH• radical solution (250 μL) was added. The plates were kept in dark at room temperature for 30 min. Absorbance was measured at 517 nm.

B. 2,2 -Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

ABTS (9.7 mg) was dissolved in distilled water (2.5 mL) to obtain 7 mM ABTS solution (Re *et al.*, 1999;

Lalhminghlui & Jagetia, 2018). $K_2S_2O_8$ (37.5 mg) was dissolved in distilled water (1 mL) to obtain 140 mM $K_2S_2O_8$ solution. ABTS solution (2.5 mL) was mixed with $K_2S_2O_8$ solution (44 µL), and then stirred at room temperature in dark for 15 hr. One mL of this solution was mixed with 88 mL of 50% EtOH to obtain $ABTS^{\bullet+}$ solution. The methanolic solutions of Gallic acid standard and chalcones (50 µL) were loaded in 96 well plates in triplicates, and then $ABTS^{\bullet+}$ solution (250 µL) was added. The plates were kept in dark at room temperature for 30 min. Absorbance was measured at 734 nm.

C. Nitric oxide (NO) assay

In this assay, nitric oxide radical ($NO^{\bullet}$) was generated from sodium nitroprusside and it was measured by the Griess reaction (Tsikas, 2007). Briefly, 10 mM sodium nitroprusside, phosphate-buffered saline (prepared by mixing 50 mL of 0.2 M KH_2PO_4 with 39.1 mL of 0.2 M NaOH) and Griess reagent (prepared by mixing 0.66 mL of H_3PO_4, 0.25 g of sulphanilamide and 0.025 g of α-naphthylenedihydrochloride in 25 mL of distilled water) were prepared. The methanolic solutions of Gallic acid standard and chalcones (10 µL) were loaded in 96 well plates in triplicates, and then were added 10 mM sodium nitroprusside (40 µL) and phosphate buffer saline (10 µL). The plates were incubated at room temperature for 2.5 hr. Now, the Griess reagent (40 µL) was added to each well and then incubated for another 30 min. Absorbance was measured at 550 nm.

Calculation

The absorbance values obtained in spectrophotometry were used to calculate % inhibition at different concentrations employing following equation:

$$\text{Inhibition (\%)} = \left(1 - \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}}\right) \times 100 \quad \ldots\ldots(1)$$

where, A_{blank} = absorbance of methanol blank, A_{sample} = absorbance of sample solution together with the reagent(s), and $A_{control}$ = absorbance of blank and the reagent(s) but without sample.

Microsoft excel program was used for the statistical analysis, and the results are presented with the values of standard error mean (SEM). The linear regression plots were used to calculate the IC_{50} values as given:

$$IC_{50}\ (\mu M) = \frac{(50 - c)}{m} \qquad \ldots\ldots\ldots (2)$$

where, c = intercept, m = slope of the linear curve plotted between inhibition (%) and sample concentration (µM).

RESULTS AND DISCUSSION

Butylated hydroxytoluene (BHT), butyl hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), etc. are some examples of synthetic antioxidants that used as food preservatives despite of their known negative effects. Finding of a new antioxidant that works safely in human body is always essential. We are continuously searching simple and readily available molecules that can display anticancer property effectively (Thapa *et al.*, 2016; Bajracharya *et al.*, 2020; Gupta *et al.*, 2021). In this study, we have used some (*E*)-chalcones (**1**-**7**) which were prepared through the classical Claisen-Schmidt condensation between arylmethyl ketone and aromatic aldehyde (Table 1). They were fully characterised by spectroscopies and were screened for their antioxidant capacity in scavenging of $DPPH^{\bullet}$, reducing of $ABTS^{\bullet+}$ and blocking of formation of $NO^{\bullet}$ by using the DPPH, ABTS and NO assays, respectively.

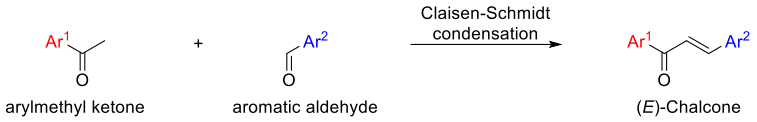
Generally, the $DPPH^{\bullet}$ free radical scavenging ability of an antioxidant is correlated with its phenolic structure which can transfer single electron or a hydrogen atom, however; the synergistic and antagonistic effects of other substituents are usually unpredictable. Furthermore, the DPPH reaction depends on the steric accessibility of the radical rather than the chemical property of the compound, therefore; the assay requires to be evaluated for a longer period (Ratnavathi & Komala, 2016). Additionally, methanol is generally used as the solvent in the DPPH assay which strongly binds H atom inhibiting HAT process (Barclay *et al.*, 1999). SET is pH-dependent, while HAT is pH-independent (Schaich *et al.*, 2015). On the other hand, *in situ* generated $ABTS^{\bullet+}$ in the ABTS assay is a chemically stable chromophore compound which tolerates a wide pH range. In earlier, the presence of an electron donating group in a chalcone has shown to possesses antioxidant activity by scavenging $DPPH^{\bullet}$ radical (Belsare *et al.*, 2010; Sivakumar *et al.*, 2011; Narsinghani *et al.*, 2013; Murti *et al.*, 2013; Díaz-Rubio *et al.*, 2019). We have also reported that both 4-OH and 3-OMe substituents in the B-ring of chalcone are apparently necessary to produce the antioxidant activity (Thapa *et al.*, 2016). In this study, we wish to learn the effect of nitrogen and oxygen heteroatom(s) (that can exchange an electron) in chalcones to produce the antioxidant effect. Thus we chose a number of simple (*E*)-chalcones to use in the DPPH, ABTS and NO assays, and the results obtained were compared with the parent chalcone (**1**) and Gallic acid standard.

From the absorbance values measured at 517 nm in the DPPH assay, a linear curve was plotted for Gallic acid standard ($y = 1.486x + 20.261$, $R^2 = 0.961$), and the IC_{50} value of 20 µM (calcd. 3.4 µg/mL) was computed, which was in close agreement with the literature value (Fig. 3) (Ghasemzadeh *et al.*, 2015; Sreedevi & Vijayalakshmi, 2018). It was not surprise to us that the parent chalcone (**1**) as well as chacones **2** and **3** bearing nitro substituent at 3'-position were ineffective to scavenge the $DPPH^{\bullet}$ free radical, at the same time, antagonism effect was realized with the remaining chalcones (**4**-**7**) although they possessed hydroxyl substituent (Table 2).

A linear curve of Gallic acid standard ($y = 0.788x + 17.680$, $R^2 = 0.971$) computed in the ABTS assay exhibited the IC_{50} value of 41 µM (calcd. 7 µg/mL) and was comparable with the literature (Fig. 4) (Biskup *et al.*, 2013; Lee *et al.*, 2015). Among the chalcones screened,

chalcone **7** (y = 0.089x + 8.452, R^2 = 0.974, IC_{50} = 464 µM, calcd. 124 µg/mL) has displayed effective antioxidant activity in the ABTS assay (Fig. 5) while remaining chalcones **1**-**6** were found insignificant (Table 2). It is worth noting that the chalcone **7** possesses *p*-dimethylamino substituent in the B ring. Hence, the presence of a *p*-dimethylamino substituent may account for its notable result. Díaz-Rubio *et al.* (2019) have mentioned that the enhanced electronic density due to electron donating group may increase the antioxidant activity. This increase can be attributed to the resonance effect involving the participation of lone pairs of electrons present on the heteroatom. Apparently, a strong electron donating group (especially amino group) at *para*-position in B ring has synergistically enhanced reduction of $ABTS^{\bullet+}$ (Table 2, compare entries 8 *versus* 7). Replacement of the phenyl ring with a furyl B ring was also found insignificant (Table 2, entries 4 and 6). Consequently, *p*-dimethylamino substituent in phenyl B ring of the chalcone has proved advantageous in scavenging cationic free radical that may help in identifying potential new drug candidates for the cancer treatment. However, the effect of *p*-dimethylamino substituent in scavenging of $NO^{\bullet}$ free radical was found irrelevant (*vide infra*).

Table 1. Claisen-Schmidt condensation to prepare chalcones.



Ar^1	Ar^2	(*E*)-Chalcone	Yield (%)	State
phenyl	phenyl	(*E*)-1,3-Diphenylprop-2-en-1-one (**1**)	82.1	Light yellow solid
3-$NO_2C_6H_4$	phenyl	(*E*)-1-(3-Nitrophenyl)-3-phenylprop-2-en-1-one (**2**)	40	Cream solid
3-$NO_2C_6H_4$	furyl	(*E*)-3-(Furan-2-yl)-1-(3-nitrophenyl)prop-2-en-1-one (**3**)	43	Yellow-cream solid
2-HOC_6H_4	phenyl	(*E*)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (**4**)	43.8	Yellow solid
2-HOC_6H_4	furyl	(*E*)-3-(Furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5**)	85.1	Yellow solid
2-HOC_6H_4	4-$MeOC_6H_4$	(*E*)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**6**)	70	Yellow solid
2-HOC_6H_4	2-$NMe_2C_6H_4$	(*E*)-3-(4-(Dimethylamino)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**7**)	52	Red solid

The DPPH and ABTS assays are considered non-physiological resemblance due to the absence of $DPPH^{\bullet}$ (Granato *et al.*, 2018) and $ABTS^{\bullet+}$ (Magalhães *et al.*, 2008) in the human body. So, criticism has been appealed that these assays are not directly relevant to the biological function (Schaich *et al.*, 2015). On the other hand, $NO^{\bullet}$ is released from amino acid in endothelial and neuronal cells (Thomas, 2015). At low concentrations, $NO^{\bullet}$ plays effective role in antitumor effect, neuronal messenger and vasodilation, however; a high level of $NO^{\bullet}$ can cause several health complications including arthritis, colitis and sclerosis. The toxicity increases when $NO^{\bullet}$ reacts with superoxide radical. We thus further envisioned to perform the NO assay of the chalcones **1**-**7**. Gallic acid standard (y = 0.160x + 36.699, R^2 = 0.904) has displayed the IC_{50} value of 83 µM (calcd. 14 µg/mL) in the NO assay (Fig. 6), which was resembled with the literature value (Ghasemzadeh *et al.*, 2015). Although, these chalcones could scavenge the $NO^{\bullet}$ free radical up to 42% at 500 µM concentration, however; further improvement was not observed upon concentration increment (Table 2). It can be realized that a synergistic effect due to substituents is important. An electron donating group (e.g. hydroxyl) in 2'-position and an electron donating group (e.g. alkoxyl or amino) in 4-position could produce synergistic effect to scavenge $NO^{\bullet}$ free radical.

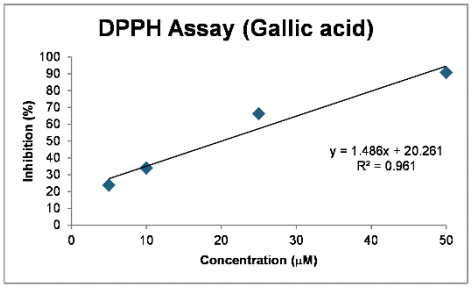


Figure 3. Inhibition of $DPPH^{\bullet}$ with Gallic acid.

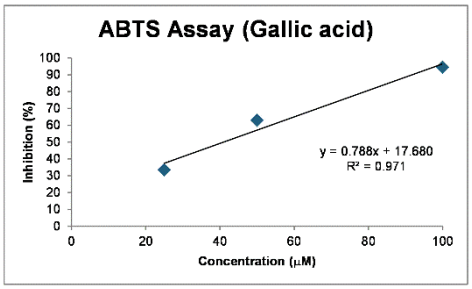
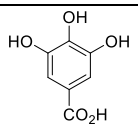
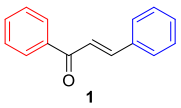
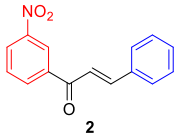
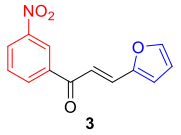
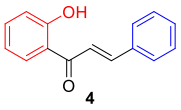
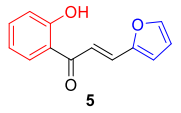
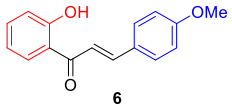
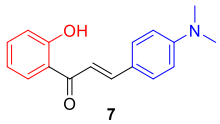


Figure 4. Inhibition of $ABTS^{\bullet+}$ with Gallic acid.

Table 2. Antioxidant activity of (*E*)-chalcones.

Entry	Compound	Inhibition % ± Standard error mean at 500 µM concentration (IC_{50})		
		DPPH	ABTS	NO
1	 Gallic acid	90.747±0.415[a] (IC_{50} = 20 µM, calcd. 3.4 µg/mL)	62.929±2.043[a] (IC_{50} = 41 µM, calcd. 7 µg/mL)	42.464±0.162[a] (IC_{50} = 83 µM, calcd. 14 µg/mL)
2	 **1**	14.828±0.345	1.402±3.738	42.650±0.323
3	 **2**	15.172±0.345	–10.903±3.978	37.828±1.159
4	 **3**	14.138±0.172	3.115±1.358	30.689±0.305
5	 **4**	10.241±0.384	16.667±7.922	8.237±1.321
6	 **5**	13.644±0.790	23.972±0.164	14.582±0.582
7	 **6**	12.644±0.575	15.576±2.960	29.835±1.016
8	 **7**	–12.989±1.092	62.399±3.338 (IC_{50} = 464 µM, calcd. 124 µg/mL)	28.920±0.305

[a] % Inhibition at 50 µM concentration.

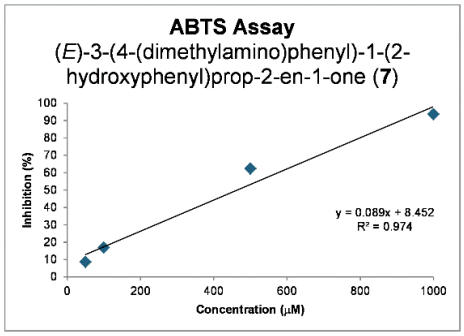


Figure 5. Inhibition of $ABTS^{\bullet+}$ with compound 7.

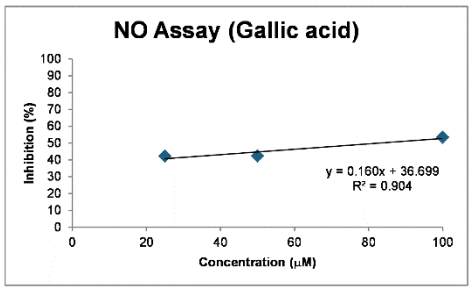


Figure 6. Inhibition of $NO^{\bullet}$ with Gallic acid.

CONCLUSIONS

Chalcones (**1**-**7**) were evaluated for the antioxidant activity by employing three different assays namely DPPH, ABTS and NO assays. (*E*)-3-(4-(Dimethylamino)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**7**) has displayed antioxidant activity by reducing $ABTS^{\bullet+}$ with IC_{50} value of 464 µM (calcd 124 µg/mL). This study has shown that other screened chalcones (**1**-**6**) were insignificant, however; this does not mean that they were completely ineffective, as antioxidant property can be relied on various mechanisms. Therefore, it is worthy to synthesize a large number of chalcones, and they should be evaluated by different cell line assays. This study has also indicated that the presence of a strong electron donating group (e.g. dimethylamino group) at *para*-position of B ring of chalcone can be considered for the development of anticancer drug in the future.

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AUTHORS CONTRIBUTION STATEMENT

Laboratory works: PT & NB; Data analysis: NB & GRU; Supervision, writing manuscript: GBB.

CONFLICT OF INTEREST

The authors do not have any conflict of interest pertinent to this work.

DATA AVAILABILITY STATEMENT

Supporting data of this study are available from the corresponding author, upon reasonable request.

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