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SYNTHETIC CURCUMINOID COMPOUNDS: AN APPRAISAL OF *IN VITRO* ANTIOXIDANT ACTIVITY

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ABSTRACT

The present study aimed at synthesizing curcumin and some related curcuminoid compounds and thereafter evaluation of these compounds for possible antioxidant activity. Previously synthesized and identified Curcumin and curcuminoid compounds were taken for the study. Antioxidant potential of the compounds were investigated. Total antioxidant activity, reducing power assay, nitric oxide scavenging activity, hydrogen and DPPH radical scavenging activity were performed to evaluate the antioxidant potential of these compounds *in vitro*. The IC_{50} values in DPPH model were also calculated. The results of the study revealed potential antioxidant activities of the evaluated compounds.

Keywords: Curcumin, Curcuminoid, Antioxidant, Free radical, Turmeric

INTRODUCTION

Turmeric has been used as a traditional and folklore medicine in India from time immemorial. It has a warm, bitter taste and is a primary component of curry powders. The dried rhizomes or tuber may be used in medicine as either a stimulant, carminative, hematic in many kinds of haemorrhages and as a remedy for certain type of jaundice and other liver problems [1]. Externally, it's applied to minor wounds and certain skin eruptions, decoction provides relief for a burning sensation in eye diseases, it is also considered very good for irregular menstruation. It enhances circulation, dissolves blood clots. Turmeric has also been prescribed as a remedy for pains in abdomen chest and the back. The current traditional Indian medicine claims the use of turmeric against biliary disorders, anorexia, diabetic wounds, hepatic disorders, rheumatism and sinusitis [2, 3]. The most impotant biologically active phytoconstituent of turmeric is curcumin. The potential of curcumin as a therapeutic moiety is enormous. Grown body of published literature suggested curcumin as a miracle molecule in near future [4-10]. Researchers throughout the world indulge in studies involving investigation of therapeutic role and mechanism of curcumin [11-14]. A huge body of reports demonstrated synthesis of newer Curcuminoid compounds and their possible biological activity [15-18]. In the light of growing research, the present study aims at the synthesis of two Curcuminoid compounds and their evaluation of antioxidant activity *in vitro*. Oxygen can be a toxic agent for living tissues in terms of free radicals or reactive oxygen species (ROS). Oxygen is found to be involved in the generation of various kinds of ROS. ROS, formed during normal physiological metabolism or through the action of ionizing radiation. ROS can interact with bio-molecules and which leads to an onset of degenerative diseases such as cancers, cardiovascular diseases (CVD) and other illnesses [10, 19]. An antioxidant defense system composed of a group of compounds and enzymes normally protects physiological system from destructive effects of ROS. Otherwise, antioxidants are to be sequestered from the diet or through supplementation. Most citrus and dried fruits, cruciferous vegetables, garlic, onions, carrots, tomatoes, Sweet potatoes, sesame and olive oil are rich sources of antioxidants. There are thousands of naturally occurring and synthetic antioxidants known. These antioxidants belong to different classes of compounds, such as carotenoids, polyphenolics,

polyarnines, gallic acid derivatives, tannins and catechins. Examples include phytic acid, lipoic acid, bilirubin, melatonin,quercctin, camosol, camosic acid, hydroxytyrosol, rutin, butylated hydroxyanisole, and butylated hydroxyl toluene. Vitamins E and Care among the most effective antioxidants with preventive effects againsts heart diseases and cancers [20].

MATERIALS AND METHODS

Curcuminoid compounds

The previously synthesized compounds considered for the present study are as follows:

- 1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Curcumin)
- 1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (Compound-1)
- 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione (Compound-2)
- 1,7-Bis(4-cholrophenyl)-1,6-heptadiene-3,5-dione(Compound-3)
- 1,7-Bis(3-nitrophenyl)-1,6-heptadiene-3,5-dione(Compound-4)
- 1,7-Bis(4-methylphenyl)-1,6-heptadiene-3,5-dione(Compound-5)
- 1,7-Bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (Compound-6)
- 1,7-Bis(4-fluorophenyl)-1,6-heptadiene-3,5-dione(Compound-7)
- 1,7-Bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (Compound-8)
- 1,7-Diphenyl-1,6-heptadiene-3,5-dione(Compound-9)
- 1,7-Bis(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (Compound-10)

In vitro antioxidant activity appraisal

Total antioxidant activity: The total antioxidant activity of the test sample was determined according to the previously described thiocyanate method [19, 21]. Test sample (10 mg) was dissolved in 10 mL water. Different concentration of test sample (50-250 µg/mL) or standard samples in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to linoleic acid emulsion (2.5 mL) in potassium phosphate buffer (0.04 M, pH 7.0). Five millilitres linoleic acid emulsion consists of 17.5g Tween-20, 15.5µl linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). On the other hand, 5.0 mL control consists of 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37˚C in a glass flask and in the dark. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (UV -1601 Shimadzu, Japan), after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides formed. These compounds oxidize Fe^{2+} to Fe^{3+}. The latter Fe^{3+} ions form complex with SCN^-, which had maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without test sample or standards were used as blank samples. All data about total antioxidant activity are the average of triplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by following equation:

Inhibition (%) = $(A_0 - A_t / A_0) \times 100$

Where A_0 was the absorbance of the control reaction and A_t was the absorbance in the presence of the sample. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. α-Tocopherol were used as standard antioxidant compound.

Reducing power: The reducing power of test sample was determined according to the method described previously [22]. The different concentrations of test sample (50-250µg/mL) in 1mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50 ˚C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer (UV -1601 Shimadzu, Japan). Higher absorbance of the reaction mixture indicated greater reducing power. α-Tocopherol was used as standard antioxidant compound.

In vitro antioxidant activity of the selected compounds

Determination of DPPH (1-1-diphenyl- 2-picryl hydrazyl) radical scavenging activity: The free radical scavenging activity of test sample was measured by DPPH• using the method described previously [23]. A 0.1mM solution of DPPH• in ethanol was prepared and 1ml of this solution was added to 3ml of test sample solution in water at different concentrations (50-250 µg/mL).The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer (UV -1601 Shimadzu, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation:

DPPH• scavenging effect (%) = $[(A_0 - A_t / A_0) \times 100]$

Where A_0 was the absorbance of the control reaction and A_t was the absorbance in the presence of the

standard sample or test sample. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. BHA was used as standard antioxidant compound.

ABTS (2, 2′ – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolorization assay: ABTS was dissolved in water to make a concentration of 7 mM. ABTS• was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in dark at room temperature for 12 – 16 h before use. For the test of samples, the ABTS• stock solution was diluted with phosphate-buffered saline 5 mM (pH 7.4) to an absorbance of 0.70 at 734 nm. After addition of 1.0 mL of diluted ABTS• to 20 µL of sample, the absorbance reading was taken 5 min after the initial mixing [24]. This activity is considered as percent ABTS• – scavenging which is calculated as follows:

% ABTS• – scavenging activity = [Control absorbance – Sample absorbance] / [Control absorbance] × 100

Assay of nitric oxide scavenging activity: The procedure is based on Greiss reaction [25, 26]. Sodium nitroprusside spontaneously generates nitric oxide at physiological pH in aqueous solution, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of test sample of the dissolved in methanol and then incubated at room temperature for 150 minutes. In the same way, a reaction mixture was prepared without the test sample but with equivalent amount of methanol was added. This served as control. After the incubation period, 0.5 ml of Greiss reagent (1 % Sulphanilamide, 2 % H_3PO_4 and 0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride) was added to the mixture. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine was measured at 546 nm. Standard solutions of ascorbic acid treated in the same way as tests with Greiss reagent served as positive control. The percentage of inhibition was calculated by using the following formula:

% Inhibition = $(A_0 – A_t / A_0) \times 100$

Where, A_0 was the absorbance of the control (without test sample) and A_t was the absorbance in the presence of the test sample. All the tests were performed in triplicate and graph was plotted with the mean ± SD values.

Hydrogen peroxide scavenging activity: Hydrogen peroxide (H_2O_2) scavenging ability of the test sample was measured using a method described previously [27]. A solution of hydrogen peroxide (2mmol/L) was prepared in phosphate buffer (pH 7.4). Concentration of hydrogen peroxide was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81 $(mol/L)^{-1}cm^{-1}$.The test sample (50-250 µg/ml) were added to H_2O_2 solutions (0.6 ml). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage inhibition was calculated using the following formula:

% Inhibition = $(A_0 – A_t / A_0) \times 100$

Where, A_0 was the absorbance of the control (without test sample) and A_t was the absorbance in the presence of the test sample or standard. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. Ascorbic acid was used as standard compound.

Hydroxyl radical scavenging: The reaction mixture containing 2 – deoxy – d – ribose (1 mM), phenyl hydrazine (0.2 mM), (in phosphate buffer, pH 7.4) and different concentration of RG – M (50 – 250 µg/ml) were incubated for 4 h at 37°C. The reaction was stopped by the addition of 2.8% (w/v) trichloroacetic acid solution, followed by centrifugation at 5000 rpm (for 10 min). The supernatant was mixed with aqueous 1% (w/v) thiobarbituric acid (TBA). The TBA reactive product thus formed was directly measured at 532 nm [28].

RESULTS AND DISCUSSION

In vitro antioxidant appraisal

Total antioxidant activity determination in linoleic acid system: Thiocyanate method was used to evaluate the total antioxidant activity of the test sample. The test sample exhibited effective and powerful antioxidant activity at a concentration of 250 µg/ml. The effect of 250 µg/ml concentration of the test sample on peroxidation of linoleic acid emulsion is shown in figure 45. The antioxidant activity of the test sample initially was increased with an increasing time of incubation and then it showed a decrease in activity further with increasing time of incubation. The studied concentration of the test sample exhibited higher antioxidant activity than 250 µg/ml concentration of α-Tocopherol but lower antioxidant activity than same concentration of BHA (Butylated hydroxyanisole). The percentage inhibition of peroxidation of all the compounds along with the standards in linoleic acid system was depicted in **figure 1.**

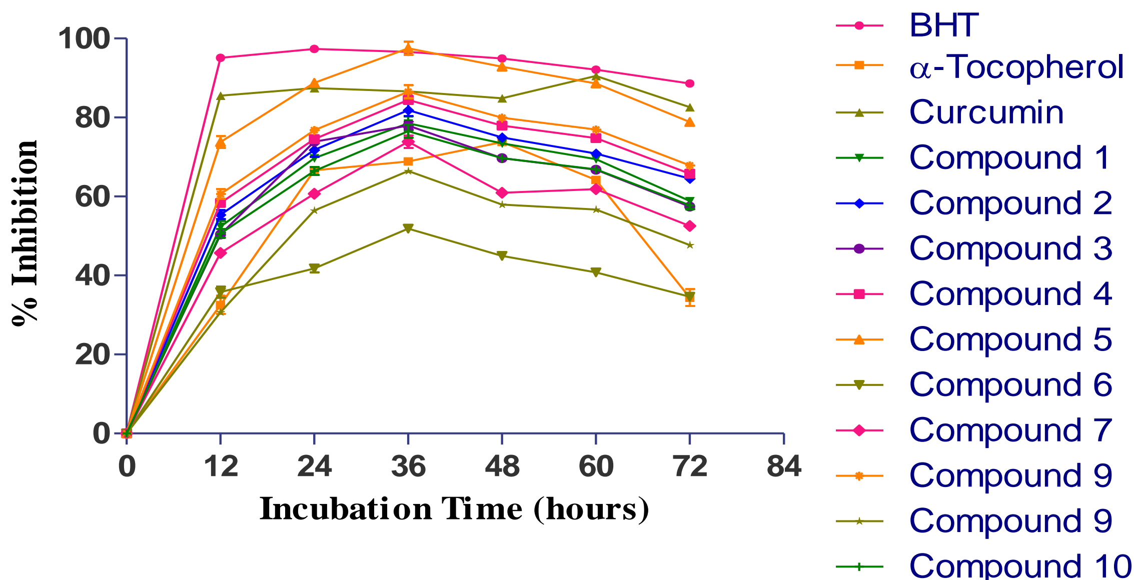

Figure 1. Total Antioxidant Activity

Effect on reducing power assay: The reducing power of the test sample compared to BHT and α-Tocopherol are shown in figure 46. In the reductive ability measurement, Fe^{3+}- Fe^{2+} transformation in the presence of test sample samples was investigated using the method of Oyaizu [22]. The reducing power of the test sample was increased with increasing concentration of the test sample. At all the studied concentrations, the test sample revealed higher reducing power than α-Tocopherol but reductive capability was lower than BHT. Reducing power of the compounds was depicted in **figure 2**.

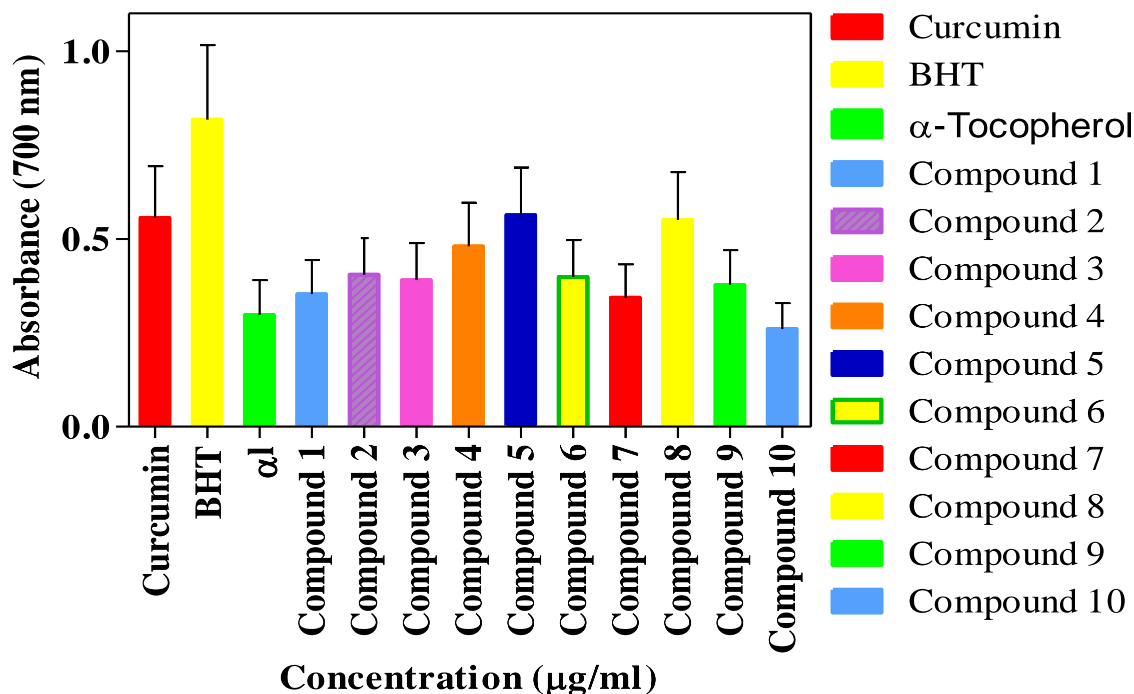

Figure 2. Reducing Power Assay

Effect of selected compounds in various models of in vitro antioxidant activity: Five compounds as selected from preliminary screening by using total antioxidant activity and reducing power assay, were subjected to DPPH radical scavenging assay, ABTS radical scavenging assay, hydrogen peroxide scavenging assay and hydroxide radical scavenging activity. The results revealed significant antioxidant activity of these compounds in the tested models as mentioned. The results are depicted in the figure 3, 4, 5, and 6.

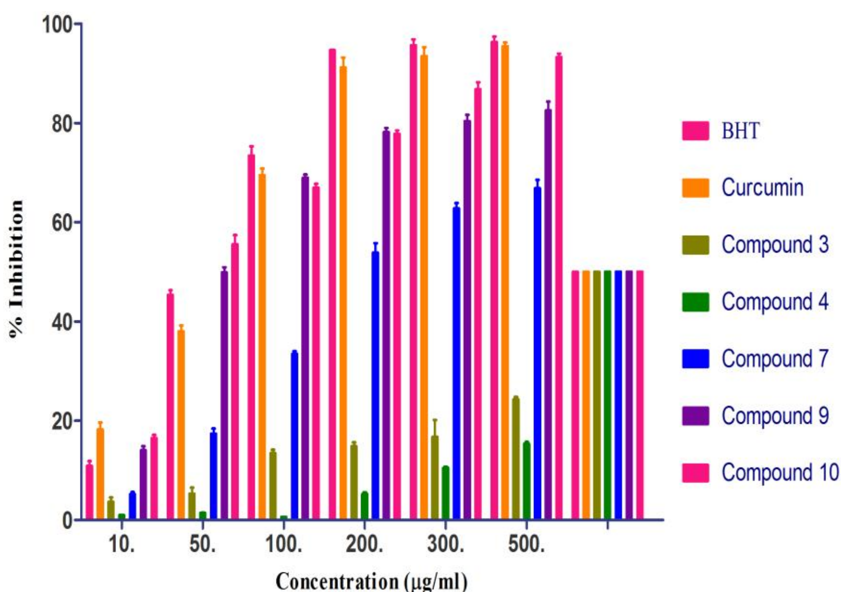

Figure 3. DPPH radical scavenging activity

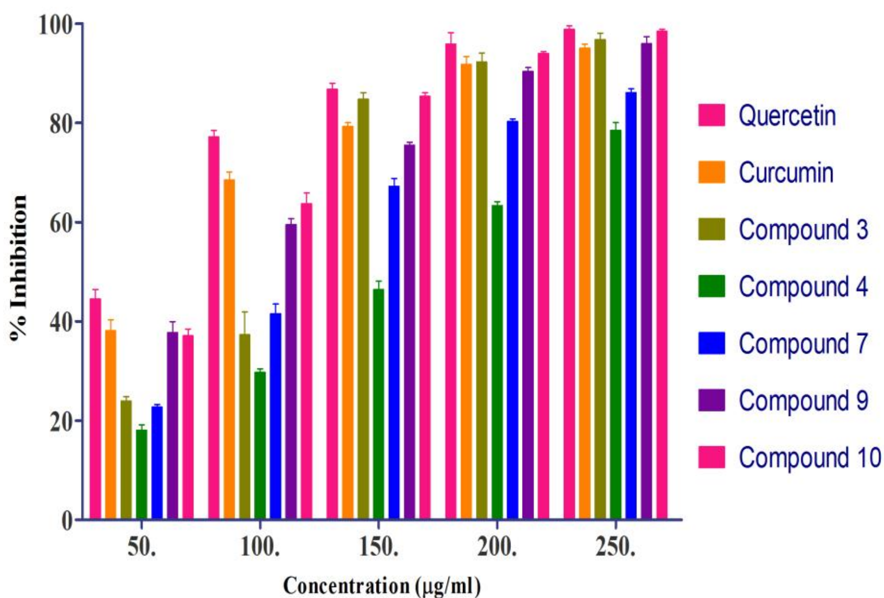

Figure 4. ABTS radical scavenging activity

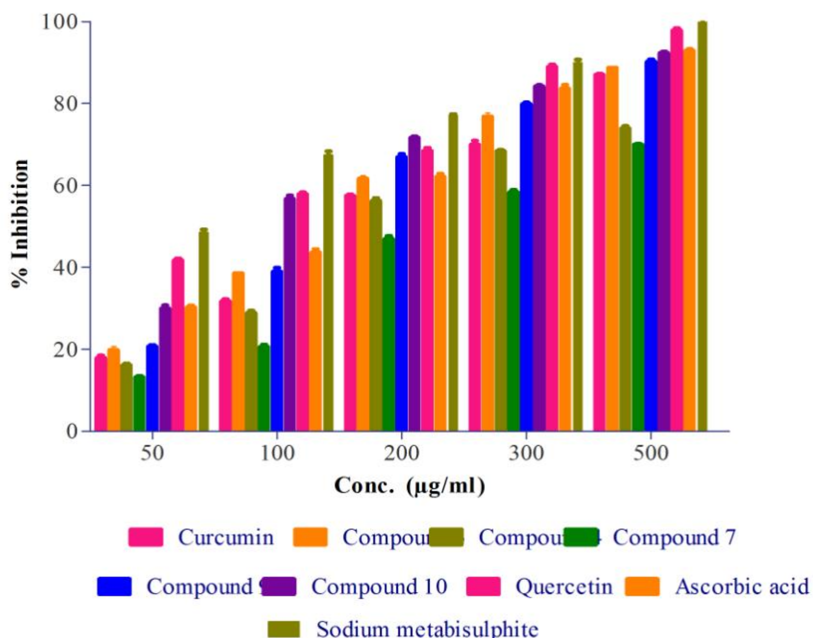

Figure 5. Hydroxyl radical scavenging activity

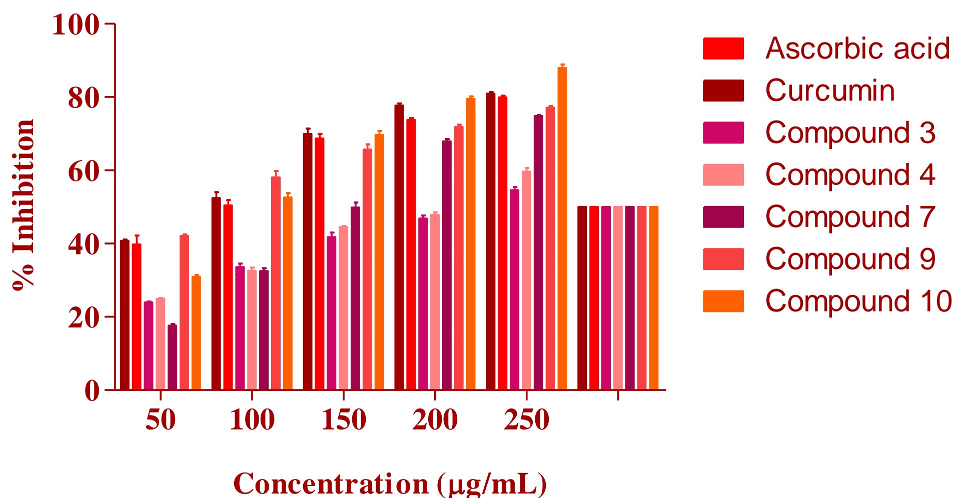

Figure 6. Nitric oxide scavenging activity

CONCLUSION

The results of present study revealed a potential antioxidant activity of ten Curcuminoid compounds. Moreover, all the compounds were found to be potent free radical scavenger *in vitro*. But five compounds which were selected from the preliminary screening were found to be significantly potent antioxidant *in vitro*. The present work demonstrated a potential way for exploring the biological activity of these compounds.

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