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# Synthesis and antioxidant appraisal of curcumin and two curcuminoid compounds

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**ABSTRACT**

*The present study aimed at synthesizing curcumin and two curcuminoid compounds and further evaluation of these compounds for possible antioxidant activity. Curcumin and two curcuminoid compounds were synthesized as per a scheme and identified through structure elucidation using instrumental techniques like NMR spectroscopy, Mass spectrometry and FTIR spectroscopy. The synthesized compounds were further screened for antioxidant potential. Total antioxidant activity, reducing power assay and DPPH radical scavenging activity were performed to evaluate the antioxidant potential of these compounds in vitro. The $IC_{50}$ value in DPPH model was also calculated. The results of the study demonstrated successful synthesis of curcumin and two curcuminoid compounds and also revealed potential antioxidant activities of the synthesized compounds.*

**Keywords:** Curcumin, Curcuminoids, Antioxidant, Turmeric, Free radicals

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## INTRODUCTION

Oxygen is an essential for life to perform biological functions like catabolism of fats, proteins and carbohydrates in order to generate energy for growth, development and other metabolic activities. However, a dark role of oxygen as a potentially toxic agent for living tissues has also been discovered. Oxygen is not dangerous by itself, but it is involved in the generation of various kinds of "reactive oxygen species" (ROS). ROS can be formed during body's normal metabolism functions or through the action of chemicals and ionizing radiation, can interact with bio-molecules and ultimately lead to an onset of degenerative diseases such as cancers, inflammation, cardiovascular diseases (CVD) and other illnesses (1, 2). Nature has created an antioxidant defense system composed of a group of compounds and enzymes efficient enough to remove free radicals before they cause tissue damage to protect against the destructive damage of free radicals. Some antioxidants are generated in the physiological system while others must be sequestered from the diet or through supplementation. Most citrus and dried fruits, garlic, onions, carrots, tomatoes, sweet potatoes, cruciferous vegetables, sesame and olive oil are rich sources of antioxidants. There are many naturally occurring and synthetic antioxidants known. Different classes of compounds, such as carotenoids, polyphenolics, polyarnines, gallic acid derivatives, tannins and catechins qualifies to be antioxidant. Some examples include phytic acid, lipoic acid, bilirubin, melatonin,quercctin, camosol, camosic acid, hydroxytyrosol, rutin, butylated hydroxyanisole, and butylated hydroxyl toluene. Vitamins E and C are among the most effective antioxidants with preventive profile against various diseases including heart diseases and cancers (3).

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 (4). Externally, its 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 (5, 6). 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 (2, 7-12). Researchers throughout the world indulge in studies involving investigation of therapeutic role and mechanism of curcumin (13-16). A huge body of reports demonstrated synthesis of newer Curcuminoid compounds and their possible biological activity (17-20). 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*.

## MATERIALS AND METHODS

### Chemistry

The compounds were synthesized as per the scheme depicted in figure 1.

### Chemicals and Reagents

Boric anhydride ($B_2O_3$) was used because it form boron complex to avoid knoevenagel condensation of the reactive methylene group (C-3) in acetyl acetone, thus making the lateral methyl groups to react with the aldehyde group of 4-hydroxy-3methoxy benzaldehyde. n - butylamine ($C_4H_9NH_2$ was used as a proton extracting reagent which activates the methyl group in 2,4- pentanedione. Tributyl borate $[CH_3 (CH_2)_3O]_3B$ was used to absorb the water that produced during the reaction. Ethyl acetate ($CH_3COOC_2H_5$) was used as a reaction solvent. Sodium sulphate (anhydrous) ($Na_2SO_4$) was used to remove water in organic solvent portion of the extraction.

### General Procedure for the Synthesis of Compounds (1-3)

2,4-pentanedione (1.0 gm., 0.01 mol) and boric anhydride (0.49 gm., 0.007 mol) were dissolved in ethyl acetate (10 mL) and stirred for 0.5 hr. at 40 $^0$C. The corresponding substituted benzaldehyde (0.02 mol) and tributyl borate (4.6 0.02 mol) were added, and the reaction mixture was stirred for 0.5 hr. after the dropwise addition of n-butylamine (1.1 gm. 0.015 mol) in ethyl acetate (10 mL) over a period of 15 min., the mixture was stirred for a further 24 hrs. at 40 $^0$C. The mixture was hydrolyzed by the addition of 10% HCl (10 mL) and heating at 60 $^0$C for 1 hr. The organic layer was separated, and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with water and dried over $Na_2SO_4$. Evaporation of the solvent left a yellow power which was purified by column chromatography (silica gel, cyclohexane, $CHCl_3$, ethyl acetate, methanol).

### Column Chromatography

The column was packed with silical gel 60-120 mesh for column chromatography in hexane. The eluting solvent first was hexane it was then changed to chloroform: hexane (25mL: 75mL), (1: 3). Elution of the desired product was still slow so that the concentration was again changed to chloroform: hexane (50: 50) this did not work well enough and it was adjusted to a ratio of 3: 1 chloroform: hexane (75mL: 25mL). Elution was still slow and at last pure chloroform (100%) was used which actually did not do much improvement and so to raise the polarity the concentration of eluting solvent was adjusted to chloroform: methanol 1% and elution of the desired product was still slow, finally chloroform: methanol 2% gave good elution whereby a series of fractions were obtained and checked through TLC. Fractions which did not show any traces of impurities so they were taken and concentrated as the product.. Spectroscopic analysis was done on the recrystallize product. Recrystalization was done with methanol.

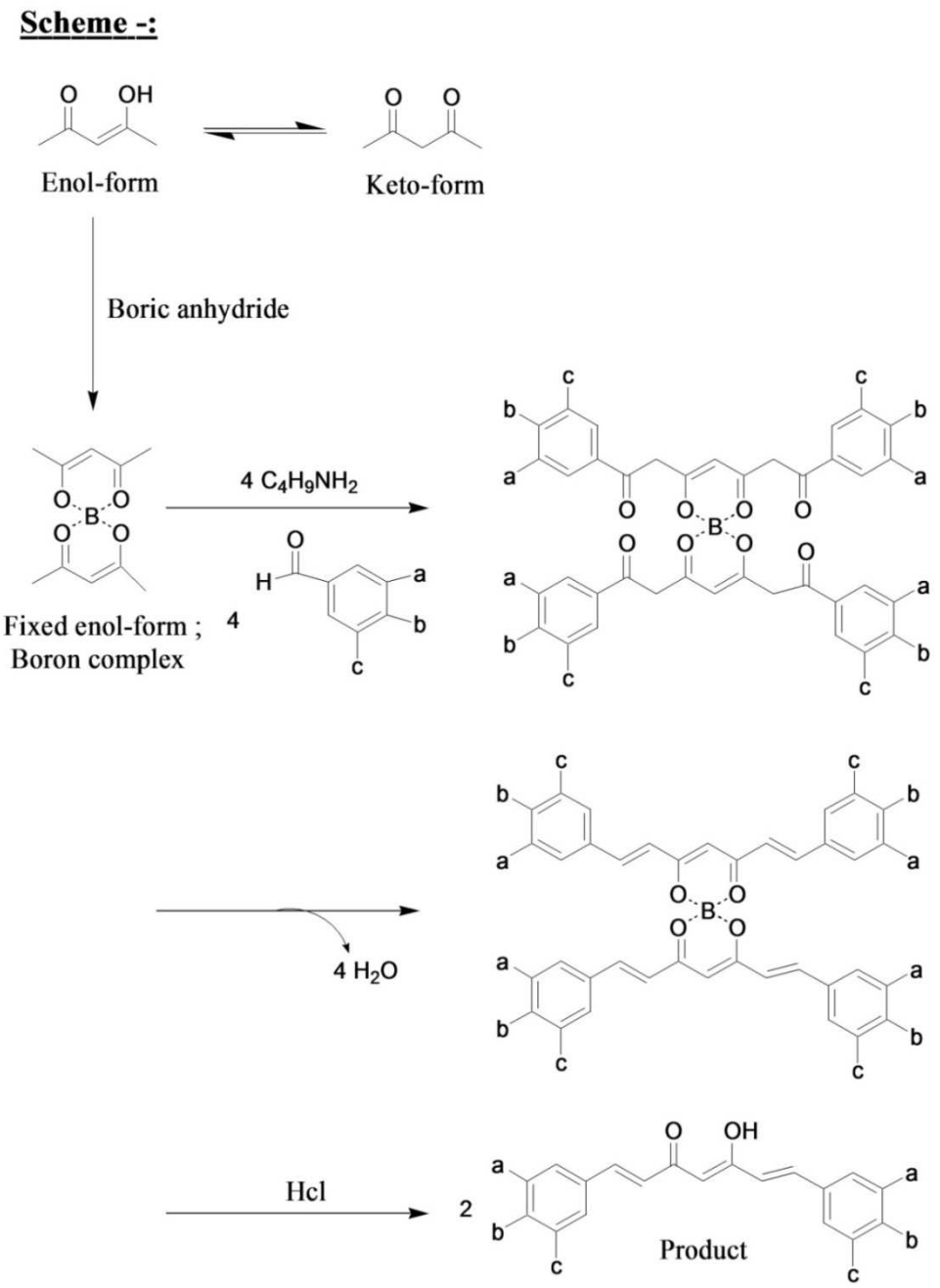



**Figure 1. Scheme for the synthesis of Curcuminoid compounds**

| S. No. | a | b | c |
|---|---|---|---|
| **1** | H | OH | OCH3 |
| **2** | H | OH | H |
| **3** | H | OH | OH |

***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 (1, 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 milliliters linoleic acid emulsion comprises 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 includes 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04 M, pH 7.0). The mixture was incubated at 37°C in a glass flask and in a dark place. The mixture was stirred for 3 min and then the

peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (UV -1601 Shimadzu, Japan), following reaction with $FeCl_2$ and thiocyanate at intervals during incubation. Peroxides formed during the linoleic acid oxidation. These compounds oxidize $Fe^{2+}$ to $Fe^{3+}$. The latter $Fe^{3+}$ ions complexes with $SCN^-$, which had maximum absorbance at 500 nm. Therefore, high absorbance suggested high linoleic acid oxidation. The solutions without test sample or standards were used as blank. All data about total antioxidant activity are the average of triplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by using the 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 utilized as standard antioxidant compound.

**Reducing power**

The reducing power of test sample was determined according to the method described previously (22). The test sample in a range (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 solution was incubated at 50 ˚C for 20 min. A part (2.5 mL) of trichloroacetic acid (10%) was added to the mixture. The mixture was then centrifuged for 10 min at 3000 rpm. The supernatant layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm in a spectrophotometer (UV -1601 Shimadzu, Japan). Higher absorbance of the reaction mixture suggested greater reducing power. α-Tocopherol was utilized as a standard antioxidant compound.

**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 solution of DPPH• (0.1mM solution of DPPH•) in ethanol was prepared and 1mL of the solution was added to 3 mL of test sample solution in water at different concentrations (10-250 µg/mL).The mixture was agitated vigorously and allowed to stand for 30 min at room temperature. Then the absorbance was measured out at 517 nm in a spectrophotometer (UV -1601 Shimadzu, Japan). Lower absorbance of the reaction mixture denoted higher free radical scavenging activity. The percent DPPH scavenging effect was calculated by the following equation**:**

$DPPH^{\bullet}$ 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 executed in triplicate and graph was plotted with the mean ± SD values. BHA was the standard antioxidant compound.

## RESULTS AND DISCUSSION

**Physical and spectral data of the synthesized compounds**

**Compound 1**

**1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione(curcumin) (Compound-1): 47% yield** from vanillin (3.04 g, 0.02 mol). **m.p. 180-181$^0$C** (lit. m.p. 182-183 $^o$C). **$^1$H NMR** [ 300 MHz, $CDCl_3$]: δ 3.9560 (s, 6H, 2 x $_3$HCO-$C_6H_4$- ), 5.8076 (s, 1H, =$CH_a$-), 6.4568-6.5094 (d, $J_{Trans}$ = 15.78 Hz, 2H, 2 x –$CH_b$=$CH_c$-), 6.9262-6.9536 (d, J = 8.22 Hz, 2H, 2 x –$C_6H_4$-), 7.0554-7.0603 (d, J = 1.47 Hz, 2H, 2 x –$C_6H_4$-), 7.1146-7.1475 (s, 2H, 2 x –$C_6H_4$-), 7.5709-7.6234 (d, $J_{Trans}$ = 15.75 Hz, 2H, 2 x -$CH_c$=$CH_b$-). **EIMS**: m/z 369.1 $(M+1)^{+\cdot}$, 370.2 $(M+2)^{+\cdot}$, 177.0 (Base peak). **IR** (KBr, $cm^{-1}$) : 3504.3, 1628.5, 1375.8, 1314.5, 1205.7, 1117.8. From the above data we can conclude that the compound-1 may be Curcumin having structure as in figure 2d.

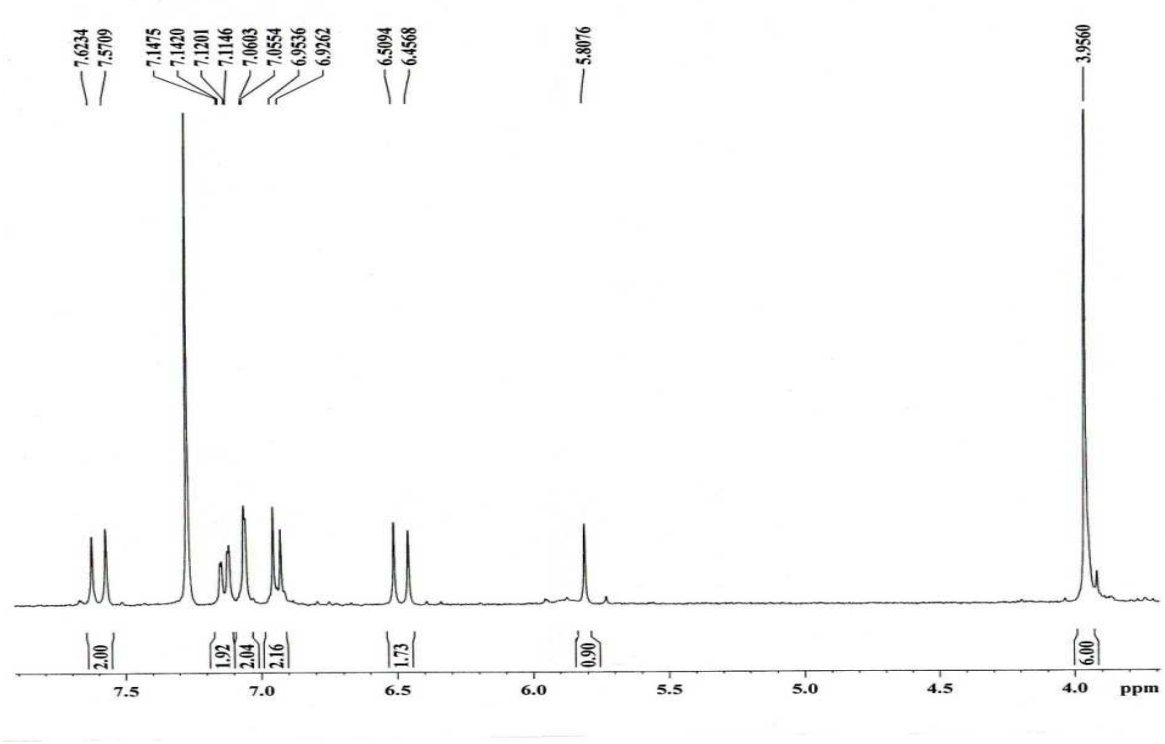



**Figure 2a. NMR spectrum of Compound-1**

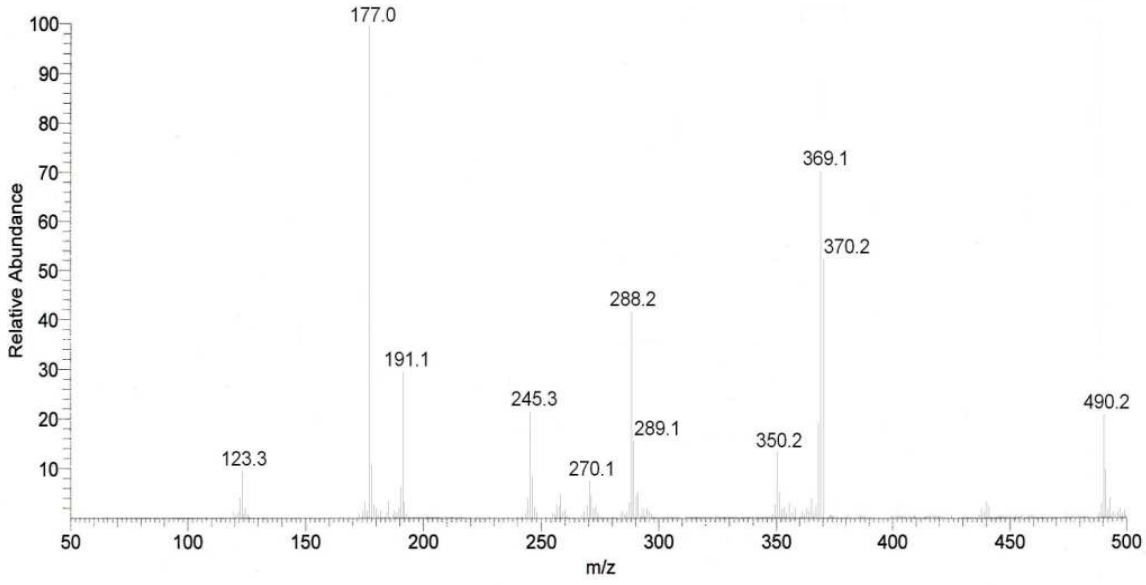



**Figure 2b. Mass spectrum of Compound-1**

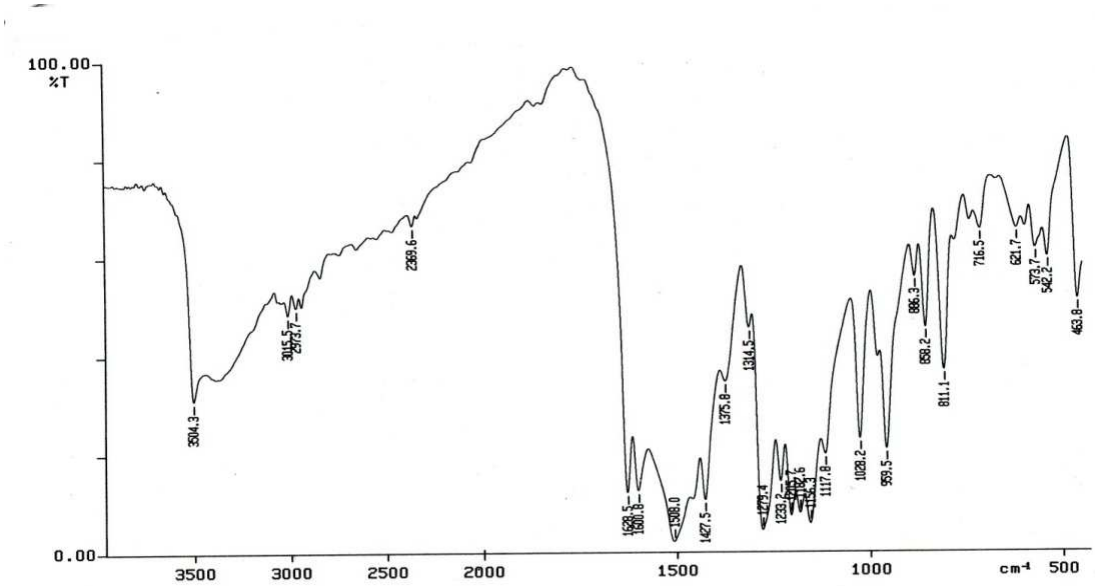



**Figure 2c. IR spectrum of Compound-1**

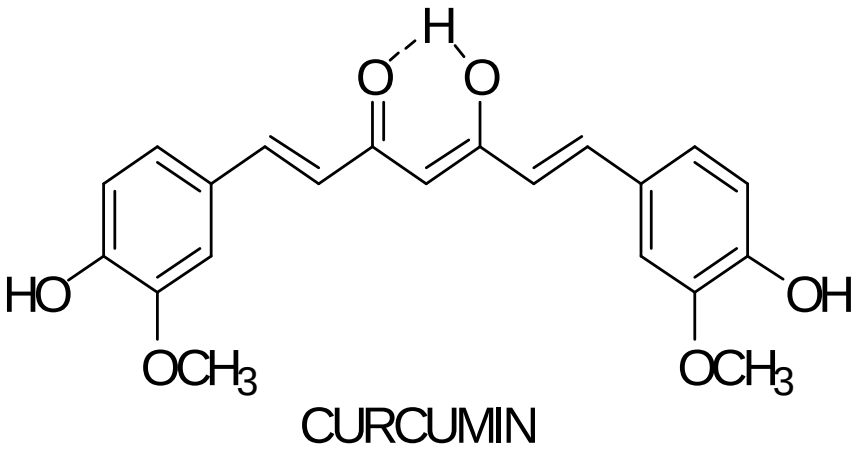



**Figure 2d. Structure of compound 1.**

**Compound-2**

**1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (Compound-2): 52% yield** from 4-hydr-oxybenzaldehyde (2.4 g, 0.02 mol). **m.p. 221-223$^0$C** ( lit. m.p. 224 $^o$C). **$^1$H NMR** [ 300 MHz, $CDCl_3$]: δ 5.8176 (s, 1H, =$CH_a$-), 6.5175-6.5694 (d, $J_{Trans}$ = 15.57 Hz, 2H, 2 x –$CH_b$=$CH_c$-), 6.9724-7.0101 (m, 4H, 4 x –$C_6H_4$-), 7.3486-7.3942 (m, 4H, 4 x –$C_6H_4$-), 7.5479-7.5998 (d, $J_{Trans}$ = 15.57 Hz, 2H, 2 x -$CH_c$=$CH_b$-). **EIMS**: m/z 308.0 $(M)^{+\cdot}$, 309.2 $[(M+1)^{+\cdot}]$, 176.3 [base peak]. **IR** ( KBr, $cm^{-1}$) : 3213.0, 1702.1, 1441.3, 1340.5, 1269.2, 1139.1. From the above data we can conclude that the compound may be 4-Hydroxy Curcuminoid having structure as in figure 3d.

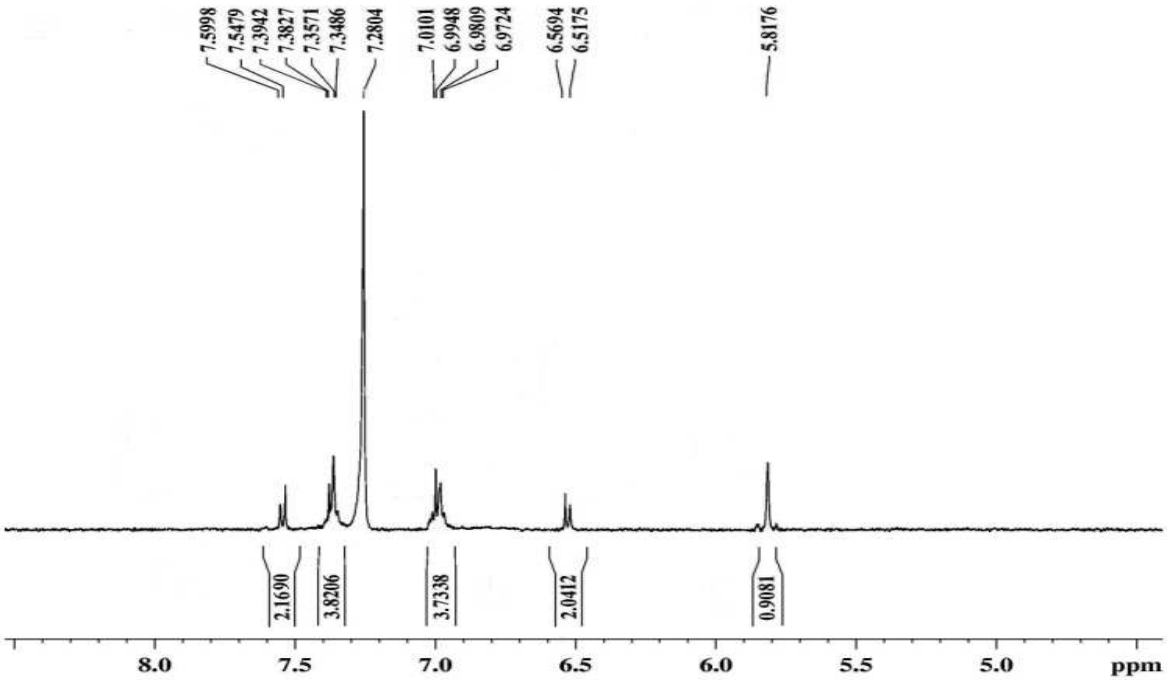



**Figure 3a. NMR spectrum of Compound-2**

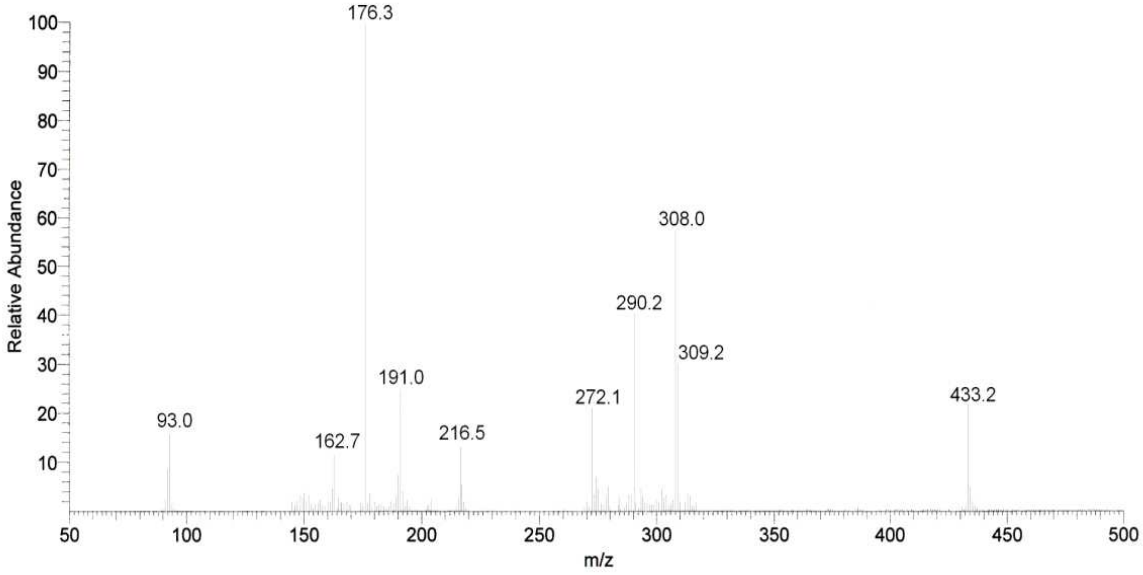



**Figure 3b. Mass spectrum of Compound-2**

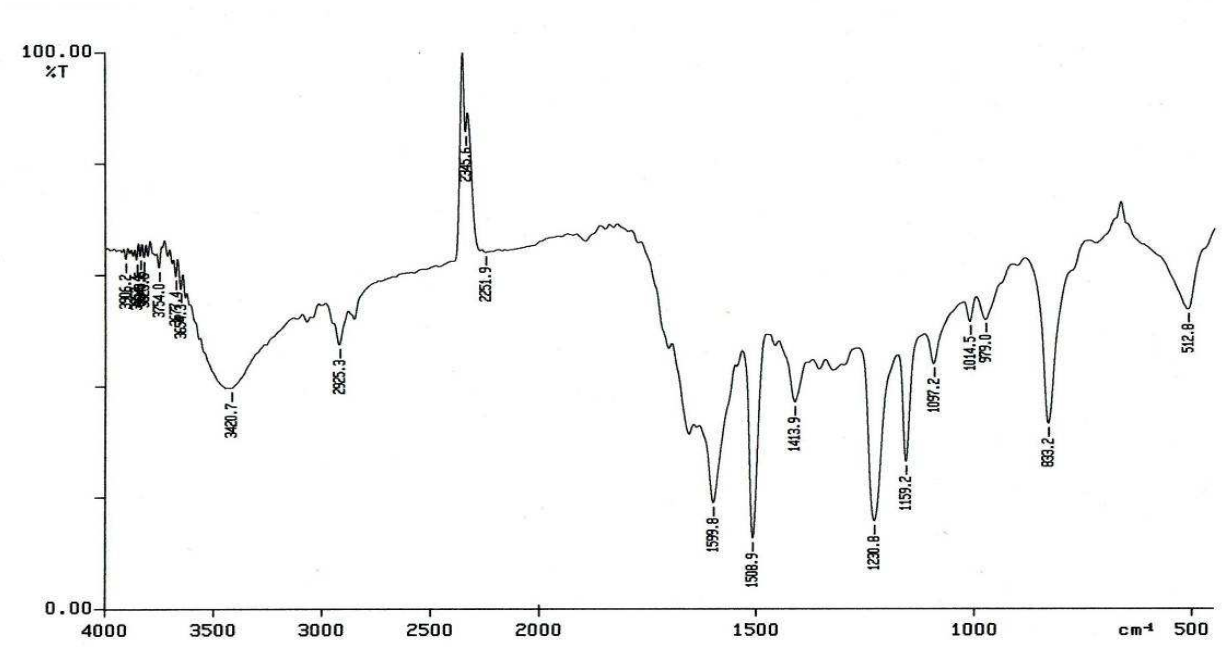
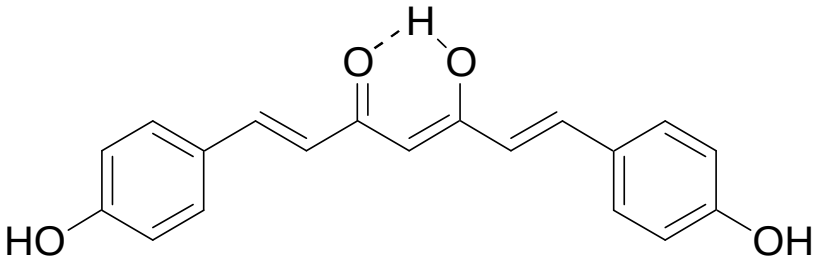



**Figure 3c. IR spectrum of Compound-2**




4-Hydroxyphenyl Curcuminoid

**Figure 3d. Structure of Compound-2.**

**Compound-3**

**1,7-Bis(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (Compound-3): 40% yield** from 3,4-dihydroxybenzaldehyde (2.76 g, 0.02 mol). **m.p. 304-306$^0$C** (lit. m.p. 306-308 $^o$C). **$^1$H NMR** [ 300 MHz, $CDCl_3$]: δ, 5.8397 (s, 1H, $=CH_a$-), 6.2737-6.3267 (d, $J_{Trans}$ = 15.9 Hz, 2H, 2 x $-CH_b=CH_c$-), 6.8590-6.8864 (d, J = 8.22 Hz, 2H, 2 x $-C_6H_4$-), 7.0032- 7.0306 (d, J = 8.22 Hz, 2H, 2 x $-C_6H_4$-), 7.0710-7.1018 (s, 2H, 2 x $-C_6H_4$-), 7.4068-7.4598 (d, $J_{Trans}$ = 15.9 Hz, 2H, 2 x $-CH_c=CH_b$-). **EIMS**: m/z 340 [$M^{+\cdot}$], 339.2 $(M-1)^{+\cdot}$ , 289.2 [base peak]. **IR** ( KBr, $cm^{-1}$) : 3234.5, 1651.6, 1445.0, 1388.7, 1298.0, 1192.2, 1117.9. From the above data we can conclude that the compound may be 3, 4-Dihydroxy Curcuminoid having structure as in figure 4d.

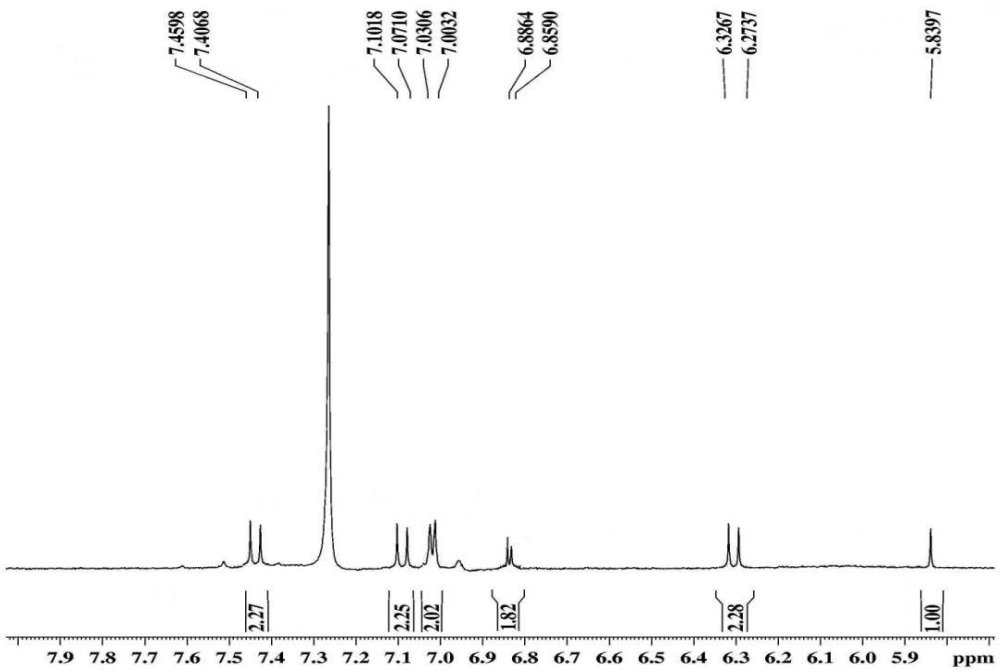



**Figure 4a. NMR spectrum of compound-3**

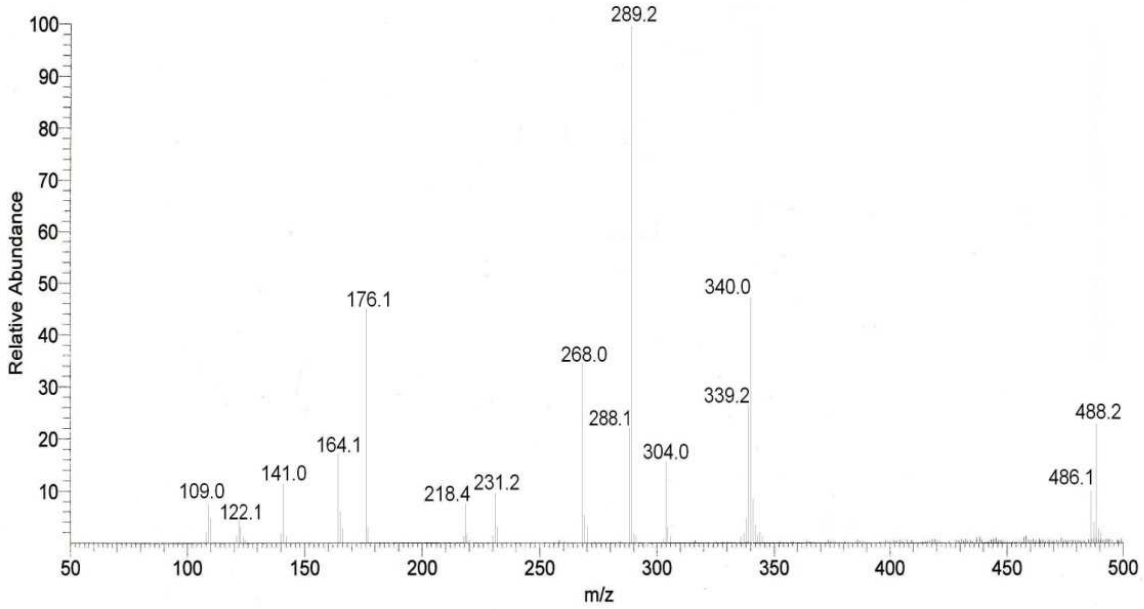



**Figure 4b. Mass spectrum of Compound-3**

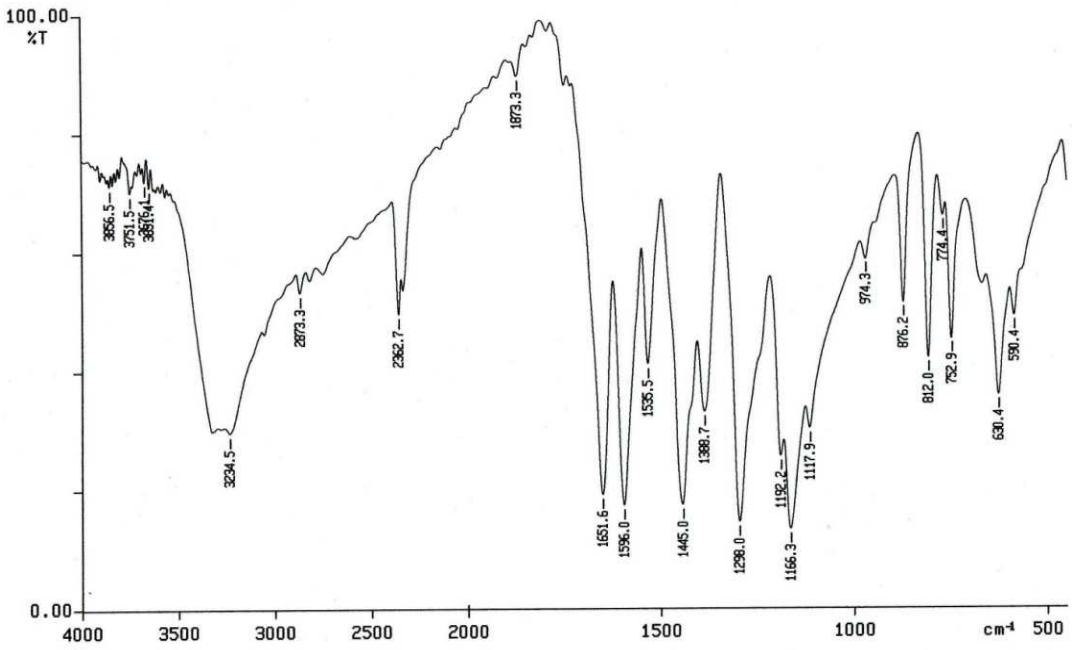
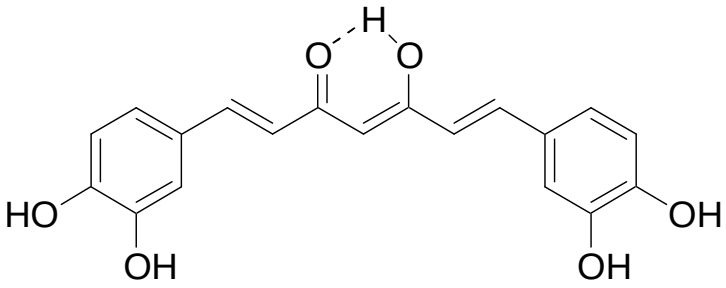



**Figure 4c. IR spectrum of Compound-3**




3,4-Dihydroxyphenyl Curcuminoid

**Figure 4d. Structure of Compound-3**

***In vitro* antioxidant appraisal**

**Total antioxidant activity**

Thiocyanate method is a widely endorsed method used to evaluate the total antioxidant activity of test sample. In the present setup 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 5. The antioxidant activity of the test sample initially was found to be increased with an increasing time of incubation and then it demonstrated a decrease in activity further with increasing time of incubation. The studied concentration of the test sample suggested higher antioxidant activity than 250 µg/mL concentration of α-Tocopherol but antioxidant activity was found to be lowered than same concentration of BHA (Butylated hydroxyanisole). The percentage inhibition of peroxidation of the compound 1, 2 and 3 in linoleic acid system was found to be 63.34 ± 1.46, 52.34 ± 1.47 and 73.78 ± 1.49 %, respectively. And percentage inhibition of 250 µg/ml concentration of BHA and α-Tocopherol was found as 95.13 ± 0.57 and 32.58 ± 2.32 %, respectively.

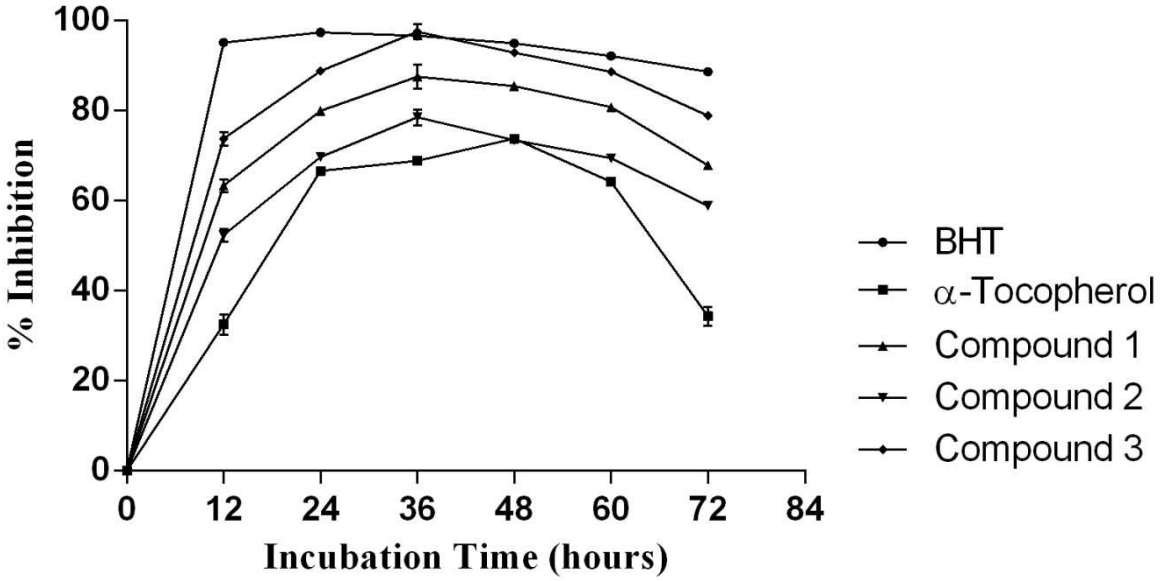



**Figure 5. Total antioxidant activity of the Curcuminoid compounds.**

**Reducing power assay**

The results of the reducing power assay of the test samples compared to BHT and α-Tocopherol are depicted in the figure 6. In the reducing power assay, $Fe^{3+}$- $Fe^{2+}$ transformation in the presence of test samples were investigated using the method of Oyaizu (22). The reducing power of the test samples were found to be increased as the concentration of the test samples increases. At all the concentrations, the test samples revealed higher reducing power than α-Tocopherol but reductive capability was lower than BHT. Reducing power of compound 1 and compound 2 were found to be similar. Reducing power of the test sample and standard compounds followed the order: BHT > Compound 1 ≥ Compound 2 > Compound 3 > α-Tocopherol

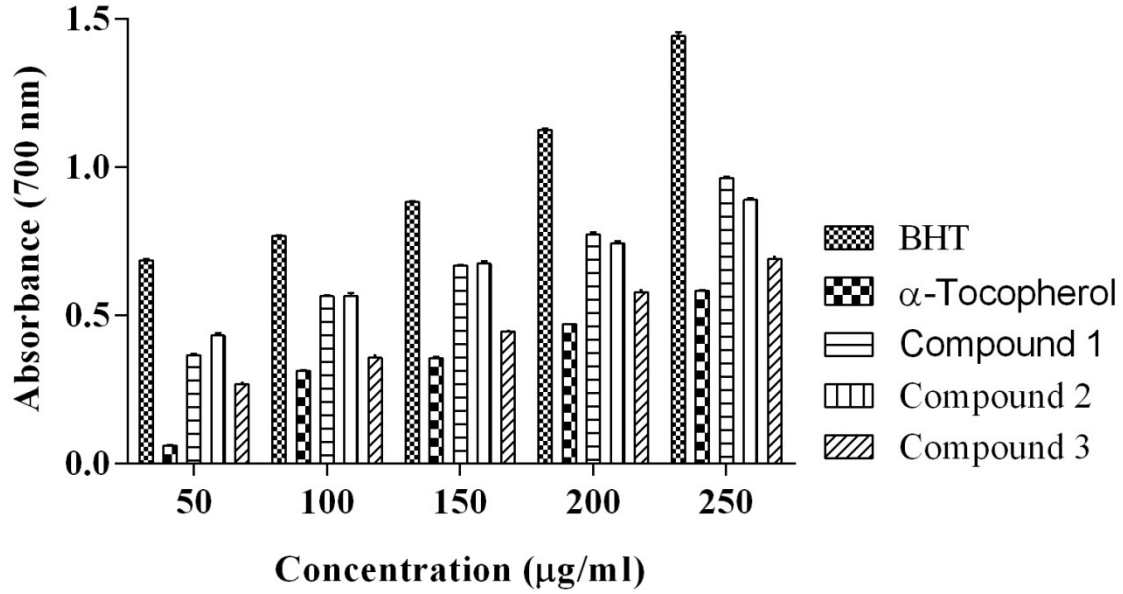



**Figure 6. Reducing power assay of Curcuminoid compounds.**

**DPPH radical scavenging activity**

Evaluation of DPPH radical scavenging activity is one less time consuming and efficient in vitro model for antioxidant activity. Evaluation of antioxidant activities by measuring scavenging capability of stable DPPH radical is a widely used method compared to other methods. As stable free radical, DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidants induced decrease in absorbance at 517 nm by scavenging of DPPH radicals. Hence, DPPH is usually as a substrate to evaluate antioxidant activity of different antioxidants whether it is of synthetic or natural origin. In this present study, BHT was a standard radical scavenger. Figure 7 depicted the decrease in concentration of DPPH radical due to scavenging ability of the test samples and standard compound (BHT) at different concentrations (10-250 µg/mL). The scavenging ability of the test samples on DPPH radical was found to be less than BHT. The percent DPPH scavenging effect of the test samples were found to be 95.47 ± 0.754, 82.503 ± 1.80, 93.243 ± 0.717 for compound 1, compound 2 and compound 3, respectively at 250 µg/mL concentration level. The percent DPPH scavenging effect the standard (BHT) was found to be 96.297 ± 1.078 at the concentration of 250 µg/mL. The results indicated all the compounds as efficient scavenger of DPPH radical comparable to standard BHT. The $IC_{50}$ values of the test compounds and BHT were calculated using the equation obtained from linear regression analysis. The calculated $IC_{50}$ values of the test compounds (Compound 1, compound 2, compound 3) and the standard compound (BHT) were found to be 74.09, 78.59 , 69.73 µg/ml and 71.03 µg/ml, respectively. When free radical formation exceeds the body's ability to protect itself, oxidative stress occurs and forms the biological basis of chronic condition (24). Data from this present study indicated that the curcuminoid compounds are efficient free radical scavenger, which can reduce or reverse the damage caused by free radicals in the human body. The compound 3 has been found to be the most powerful scavenger among all the studied compounds as indicated by lowest $IC_{50}$ value.

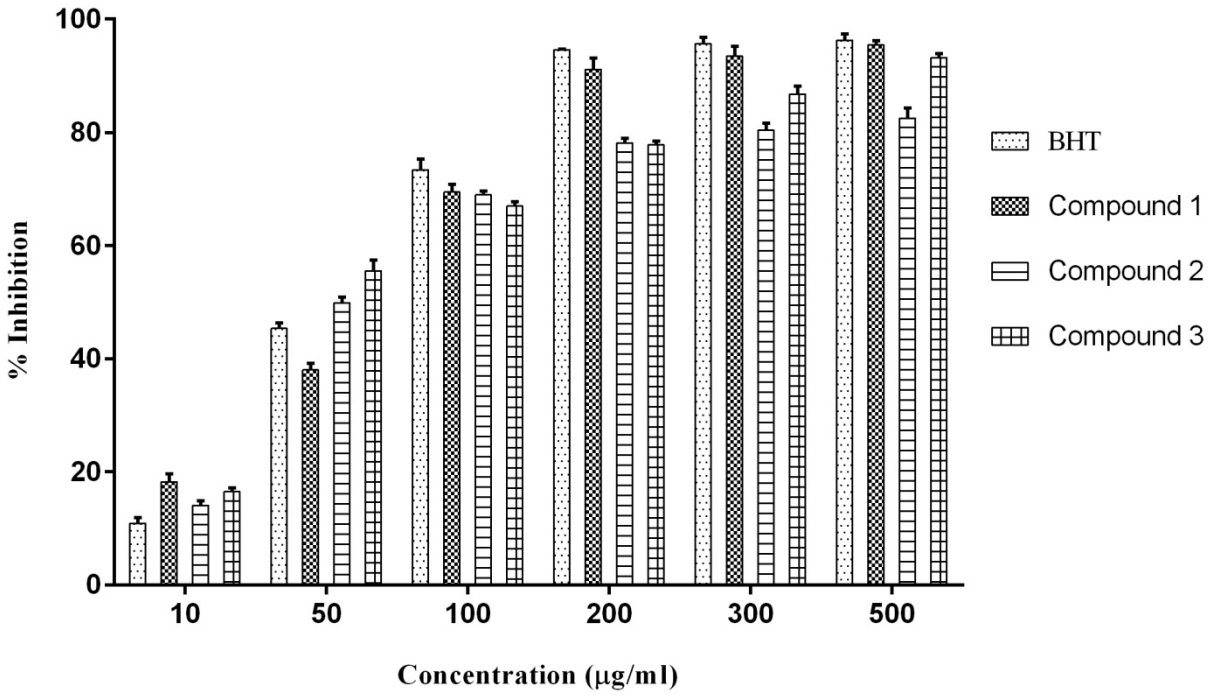



**Figure 7. DPPH scavenging activity the curcuminoid compounds**

## CONCLUSION

The present study demonstrated a successful synthesis and identification of three Curcuminoid compounds. The compound 1 was nothing but curcumin, which is successful and potential investigational molecule with various biological activity Further all the compounds were found to be potentially antioxidant *in vitro*. The study paved a way for a line of synthesis for exploring the possibility of synthesis of newer Curcuminoids for use as potential curcumin like therapeutic moiety with better safety, efficacy and physiochemical feasibility.

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