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Potential role of synthetic curcumin analogs as antibacterial, antioxidant and anticancer agent

Haya Hussain[1], Shujaat Ahmad[*1], Syed Wadood Ali Shah[2], Niaz Ali[3], Abid Ullah[1], Asaf Khan[1], Shafiq Ur Rahman[1], Mehreen Ghias[2], Hamid Hussain[1], Zul Kamal[1], Shafiullah[4] and Ziaul Islam[2]

[1]Department of Pharmacy, Shaheed Benazir Bhutto University Sheringal, Dir Upper Khyber Pakhtunkhawa, **Pakistan**
[2]Department of Pharmacy, University of Malakand Chakdara, Dir Lower Khyber Pakhtunkhawa, **Pakistan**
[3]Department of Pharmacology, Institute of Basic Medical Sciences (IBMS), Khyber Medical University Peshawar, Khyber Pakhtunkhawa, **Pakistan**
[4]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, 75270 Karachi **Pakistan**

***Correspondence:** shujaat@sbbu.edu.pk, pharmacistsyed@gmail.com **Received 23-02-2021, Revised: 27-03-2021, Accepted: 03-04-2021 e-Published: 10-04-2021**

Curcumin is naturally found in the dried rhizome of Curcuma longa plant and chemically it is polyphenol compound and has enormous biological applications such as antioxidant, anticancer, antibacterial, anti-Alzheimer's disease etc. In this study we investigated *in-vitro* antioxidant, anticancer and antibacterial potentials of the synthesized curcumin analogs (H1-H2) and have showed promising antioxidant activity by scavenging DPPH and ABTS free radicals at 1000 to 62.5 µg/mL concentration. Compound (H1) showed anti-proliferative activity with IC_{50} 254.16, 262.4 and 252.11µg/mL in 1000 to 62.6 µg/mL concentration, while curcumin (H2) showed comparable anti-proliferative activity with IC_{50} of 477.8, 254.8 and 462.6µg/mL against 3 T3, HeLa and MCF-7 cell lines respectively. Antibacterial activity showed that *Proteus mirabilis, Escherichia coli, Staphylococcus aureus and Bacillus cereus* were significantly susceptible towards both synthesized curcumin analogs (H1-H2). It was concluded from this study that the synthesized compounds could be helpful in the treatment of cancer and bacterial infection by further exploring their pharmacological profile.

Keywords: Curcumin analogs, *in-vitro* antioxidant, anti-proliferative, antibacterial activity

INTRODUCTION

Curcumin is naturally found in the dried rhizome of Curcuma longa plant and chemically it is polyphenol compound (Gagliardi et al. 2018). Curcumin are the most important therapeutic compounds have extensively studied in the past few decades. In addition, curcumin gain focus of attention for their enormous biological properties, such as antioxidant, anticancer, antibacterial, anti-Alzheimer's disease, anti-carcinogenic, antiviral, and anti-inflammatory activities (Chattopadhyay et al. 2004; Endo et al. 2014; Mošovská & Petáková, 2016). Therefore, several studies evaluating their biological activities have been conducted and many valuable curcuminoids have been developed for many ailments (Kim et al. 2018). Synthetic curcumin analogs have been developed and reported as alternative to natural curcumin for many diseases (Carapina da Silva et al. 2019).

Reactive oxygen species (ROS) such as superoxide, alkoxyl, hydroxyl, peroxyl and hydroperoxyl are the free radicals formed in humans during normal metabolism (Shoaib et al. 2015b). Complex biochemical reactions and

frequent exposure to dietary xenobiotics and environmental toxicants to human body caused production of these free radicals which consequently under different pathophysiological conditions results oxidative stress (Tanvir et al. 2017). Antioxidants scavenge these free radicals and known for their extensive biological properties including antibacterial, antiviral, vasodilator effects, anti-inflammatory and anti-thrombotic activities (Shoaib et al. 2015b).

Cancer is world menace and life threatening disease characterized by uncontrolled proliferation of abnormal cells invading surrounding tissues (Gennari et al. 2007). More than half of the 20 million people belongs to developing countries of the world have been diagnosed with different types of cancers (Siegel et al. 2015). Cancer is among the major health problem in many parts of the world and in women; breast and cervical cancer is one of the most widespread cancers among women worldwide. Among South-Central Asian countries its prevalence and incidence was reported highest in Pakistan (Shoaib et al. 2019). Resistant to available antibiotics emerged as a problem and the need for the designed and development of new antibacterial agents has become the subject of ongoing research that will overcome the resistance problem (Hamed et al., 2013). Curcumin have been reported to exhibited broad spectrum antibacterial, antifungal, anti-malarial and antiviral activities. Due to their broad spectrum antimicrobial activity and safe profile of curcumin event at 12 g/day measured in human during clinical trials, the curcumin core is used as sample to develop new antimicrobials through synthesis of their various derivatives with enhance antimicrobial spectrum (Moghadamtousi et al. 2014).

Based upon the significance of curcumin as potential therapeutic agent, we investigated antioxidant, anticancer and antibacterial activities of two synthesized mono-carbonyl and di-carbonyl curcumin analogs.

MATERIALS AND METHODS

Experimental

The chemicals used in this research study were purchased from the local market and were of analytical grade Sigma Aldrich (Merck), Germany. Thin layer chromatography (TLC) silica gel plates 60 F254 were used to check the progress of reaction under UV light (254 nm). The ^{1}HNMR spectra (300 MHz) were recorded employing Bruker Varian Mercury 300 MHz FT Spectrometer, in $CDCl_3$-d. Electrothermal 9100 (Barnstead UK) apparatus were used for the determination of melting points in open capillary tubes and were uncorrected.

Synthesis of curcumin analogs (H1-H2)

Synthesis of mono-carbonyl and di-carbonyl curcumin analogs (H1-H2) were carried out using benzaldehyde and from mono and di-carbonyl ketones respectively. Briefly, to a mixture of Benzaldehyde (2 mmol) and respective ketone (1 mmol) in 2:1 ratio in cold ethanol (15 mL) and 10 mL an aqueous solution of sodium hydroxide (40%) was added using magnetic stirrer monitored by TLC for around 2 hours for most of the products until reaction was completed. Reaction catalyst was neutralized with dilute hydrochloric acid solution, filtered. The dried solid product was purified by recrystallization in ethanol or ethyl acetate (Carapina da Silva et al. 2019)

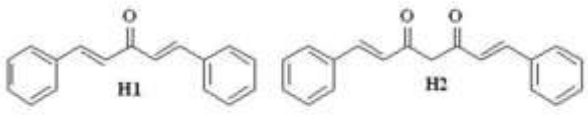

Figure: Structure of curcumin analogs (H1-H2)

(1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (H1)

Light yellow crystals, yield: 84.3%, Rf: 0.84, m.p: 109-113 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, *J* = 16.03 Hz, 2H), 7.68 (m, 4H), 7.51 – 7.37 (m, 6H), 7.13 (d, *J* = 16.01 Hz, 2H). EI MS: m/z M^{+1} 234.10 while 13CNMR, IR spectroscopy data was already reported (Dasharath Kondhare 1, 2019).

(1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione (H2)

Yellow crystalline powder, yield: 68.2%, Rf: 0.70, m.p: 151-154°C. ^{1}H NMR (300 MHz, CDCl3): δ7.69 (d, J = 15.9 Hz, 2H), 7.65 (d, J = 6.64 Hz, 4H), 7.47–7.38 (m, 6H). 6.53 (d, J = 15.7 Hz, 2H), 5.86 (s, 1H). HRMS [M]+): 276.12 while 13CNMR and IR was already reported (Hung et al. 2020).

Pharmacological activities

DPPH Radical Scavenging Activity

In-vitro antioxidant potential of curcumin analogs (H1-H2) was carried out with slight modification. A 2% DPPH (2, 2-Diphenyle-1-picrylhydrazyle) solution in methanol was prepared and I mL of this solution was added to

each 1 mL of the tested compound. After 30 minutes absorbance at 517 nm was measured and Ascorbic acid was used as standard ranging from 62.5 to 1000 µg/mL concentration. The percent scavenging activity of the compounds were calculated by the formula (Shoaib et al. 2015b):

Scavenging activity (%) = (Control Abs – Sample Abs) × 100
(Control Abs)

Where as

Control Abs = absorbance of methanol + DPPH

And Sample Abs = Absorbance of Sample/standard + DPPH

ABTS Assay

The free radical scavenging activity of compounds (H1-H2) using ABTS (2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid) as free radical was determined. The 7 mM solution of ABTS and 2.45 mM potassium persulphate (K_2S_2O4) solution were prepared, mixed and kept in dark place for 12-16 h at room temperature to obtain a dark colored solution. Dilution of this was made by adding 0.01 M Phosphate buffer pH 7.4 and at 734 nm the absorbance value was adjusted up to 0.70. The solution of synthesized compounds 300 µl was added to ABTS 3.0 ml solution in cuvette and run at 734 nm in the UV spectrophotometer (Thermo electron corporation, USA). This analysis was carried out for 6 min preceding mixing for a min and reduction in absorbance was measured. This test was repeated in triplicate and Ascorbic acid was used as a standard. The ABTS % scavenging inhibition was calculated by the formula (Zafar et al. 2019):

ABTS scavenging activity (%) = (Control Abs – Sample Abs) × 100
(Control Abs)

Anticancer activity

The cytotoxic activity of the synthesized curcumin analogs (H1-H2) was carried out using 96-well micro plate reader. In the Minimum Essential Medium Eagle (MEM) three different cell lines 3 T3, HeLa and MCF-7 were cultured and supplemented with 5% Fetal Bovine Serum (FBS), each 100 IU/mL of streptomycin and penicillin in flasks and in humidified CO_2 atmosphere (5%) at 37°C incubated. Haemocytometer was used for counting cell and were harvested and diluted with medium. The cultures of cell at densities of 5x104, 6x104 and 5x104 cells/mL for NIH 3T3, HeLa and MCF7 respectively were prepared, and added 100µl to each well into 96-well plates and pass through 24h incubation period. A fresh medium containing various concentrations 62.5 to 1000 µg/mL of test compounds was prepared. After 48 h MTT solution (200µl, 0.5mg/mL) added to well and kept for 4 h incubation. Then 100 µl DMSO (Dimethyl sulfoxide) was supplemented to each well. Level of MTT reduction to formazan was quantified at 570 nm in the cells by micro plate reader with Soft-Max Pro software (Spectra Max plus, Molecular Devices, CA, USA). Cytotoxicity of the tested compounds was recorded, % growth inhibition and IC_{50} was calculated (Shoaib et al. 2019).

Antibacterial activity

Antibacterial potential of the synthesized compounds (H1-H2) were determined using well diffusion method (Shoaib et al. 2015a) against four bacterial strains locally collected including Staphylococcus *aureus, Bacillus cereus* (Gram-positive) and *Escherichia coli,* and *Proteus mirabilis* (Gram-negative) bacteria. Agar medium 20 mL was added to each petri dish and swabbed with 10 µg/mL test microorganism and maintained for 15 min for absorption. Agar seeded petri dishes were bored 5 mm diameter through sterile Cork borer and 10 mg/mL test compound was added into each well bored. Ceftriaxone was used as standard. All the petri dishes were incubated for 24 hours at 37°C. The antibacterial potential of the synthesized curcumin analogs (H1-H2) were measured by zone of inhibition with scale.

RESULTS AND DISCUSSION

Antioxidant potential of the synthesized curcumin analogs (H1-H2)

Reactive oxygen species (ROS) such as superoxide, alkoxyl, hydroxyl, peroxyl and hydroperoxyl are the free radicals formed in humans during normal metabolism (Shoaib et al. 2015b). It is necessary to maintain balance between free radicals and antioxidant concentrations for good physiological functions (Tanvir et al. 2017). The antioxidants have specifically been used to oxidation process by removing free radical intermediates and ceases chain reactions act as reducing agents like ascorbic acid or polyphenols by oxidizing themselves (Shoaib et al. 2015b). Antioxidant compounds can scavenge free radicals and thereby can safeguard human body from free radicals (Mošovská & Petáková, 2016).

The synthesized curcumin analogs (H1-H2)

showed antioxidant activity by scavenging DPPH and ABTS free radicals at 1000 to 62.5 µg/mL concentration. Percent free radical scavenging activity against DPPH was found to be high for (H1) with IC_{50} value of 430 µg/mL comparable with (H2) which was found to be 440 µg/mL. Similarly, against ABTS compound (H1) showed free radical scavenging activity with IC_{50} of 465 µg/mL which was comparably higher than (H2) with IC_{50} 470 µg/mL. Ascorbic acid have IC_{50} value was 12.39, 8.28 µg/mL against DPPH and ABTS free radicals respectively (Table 1).

Percent free radical scavenging potentials of the synthesized compounds against DPPH were comparable than the reported DPPH free radical scavenging activity (Gounder & Lingamallu, 2012). ABTS radical assay is based on the reduction power of preformed cation radical of ABTS by antioxidant (Gounder & Lingamallu, 2012). The synthesized curcumin analogs possessed antioxidant potentials and researchers believed that curcumin with structure modification will enhance their antioxidant potentials (Anand et al., 2007), and another reason for its comparable antioxidant activity it lack the phenolic functional group in the synthesized curcumin analogs (Mošovská & Petáková, 2016).

Anticancer activity

Cancer is a global menace and considered life threatening and the second leading cause of death. Drug resistant cancers threats indicated that there is an urgent need for the development and improvement of effective anticancer drugs (Zintle Mbese, 2019). The synthesized curcumin analogs were tested on 3 T3, HeLa and MCF-7 cell lines and the results obtained (Table 2) indicted their anti-proliferative potentials. Compound (H1) is found to be active against 3 T3 cells lines with IC_{50} 254.16 and against HeLa cells 262.4 while it showed anti-proliferative potential against MCF-7 with IC_{50} value of 252.11µg/mL in 1000 to 62.6 µg/mL concentrations. Similarly, compound (H2) was found to be lower with IC_{50} values of 477.8 against 3 T3, and higher against HeLa with IC_{50} value of 254.8 and 462.6µg/mL against MCF-7 cell lines respectively. It was reported in the literature that curcumin analogs have the anticancer potentials by inhibiting cells proliferation and metastasis and raising apoptosis or cell cycle arrest in various cancerous conditions including breast and colon cancers (Zintle Mbese, 2019).

Antibacterial Activity

Antimicrobial drugs prone towards bacterial resistance day by day, and become leading problem for world population. Designed and development of new antimicrobial drugs that overcome this problem is need of time (Hamed et al. 2013). Antibacterial potential of the synthesized compounds were screened against four bacterial strains including *Bacillus cereus, Staphylococcus aureus,* (Gram-positive) and *Escherichia coli*, *Proteus mirabilis (*Gram-negative*)* bacteria and it was found that both the compounds were found to be significantly active towards the selected bacterial strains with different zone of inhibition. The results were comparable with the standard Ceftriaxone.

Table 1: DPPH and ABTS free radical scavenging assay

Scavenging activity (%)					
Samples	**Conc. µg/mL**	**DPPH**	**IC_{50} (µg/mL)**	**ABTS**	**IC_{50} (µg/mL)**
H1	1000	61.73±0.30	430	62.11±0.14	465
	500	52.38±0.38		51.14±0.03	
	250	41.29±0.33		41.28±0.15	
	125	33.27±0.45		33.13±0.11	
	62.5	24.11±0.14		24.19±0.12	
H2	1000	63.71±0.22	440	62.21±0.13	470
	500	52.12±0.13		51.17±0.14	
	250	41.28±0.24		42.23±0.19	
	125	34.13±0.14		33.12±0.13	
	62.5	26.81±0.12		26.17±0.15	
Ascorbic acid	1000	88.91 ± 1.30	12.39	97.43±1.15	8.28
	500	85.00 ± 0.30		92.65±0.70	
	250	78.76 ± 0.58		87.11±1.20	
	125	73.67 ± 0.61		83.98±0.85	
	62.5	67.74 ± 0.61		78.44±1.25	

Table 2: Anti-proliferative activity of curcumin analogs (H1-H2) against 3 T3, HeLa and MCF-7 cell line

Sample	Conc. (µg/mL)	3 T3	IC_{50} (µg/ml)	HeLa	IC50 (µg/mL)	MCF-7	IC_{50} (µg/mL)
H1	1000	61.24 ± 0.53	254.16	78.92 ± 2.83	262.4	69.32 ± 2.81	252.11
	500	56.29 ± 1.24		61.28 ± 2.61		61.63 ± 2.35	
	250	49.18 ± 1.29		47.63 ± 2.37		49.58 ± 2.05	
	125	33.03 ± 1.16		32.32 ± 2.27		31.32 ± 2.45	
	62.5	17.72 ± 1.57		22.28 ± 2.65		18.75 ± 1.36	
H2	1000	68.37 ± 2.85	477.8	74.12 ± 2.04	254.8	65.07 ± 2.04	462.6
	500	52.32 ± 2.24		57.24 ± 1.46		54.04 ± 2.14	
	250	41.26 ± 2.27		49.05 ± 1.15		41.28 ± 2.17	
	125	30.26 ± 2.35		29.12 ± 2.15		29.82 ± 1.28	
	62.5	25.52 ± 2.61		17.02 ± 2.03		16.07 ± 1.73	

Table 3: Anti-bacterial activity of curcumin analogs (H1-H2)

Zone of inhibition (mm)				
Curcumin	*P. mirabilis*	*S. aureus*	*E. coli*	*B. cereus*
H1	38±1.87	43±2.01	28±1.61	40±1.88
H2	25±1.22	39±1.71	22±1.51	35±1.71
Ceftriaxone	42±1.91	46±1.56	31±1.67	43±1.33

Data is taken as mean ± SD

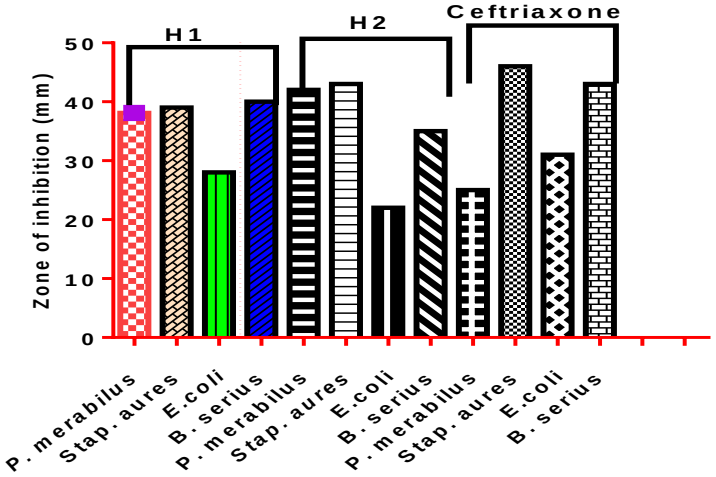

Figure 1: Antibacterial potential of curcumin analogs (H1-H2)

Curcumin (H1) was more active against *Staphylococcus aureus* and *Bacillus cereus* while *Escherichia coli* and *Proteus mirabilis* comparatively susceptible towards (H1). Compound (H2) more active against *Staphylococcus aureus* and comparatively susceptible towards others bacterial strains as shown in the Table 3 (Figure 1). The literature suggested that curcumin based compounds possessed antimicrobial activities (Hamed et al., 2013). .

CONCLUSION

In this research study two curcumin analogs were synthesized and showed antioxidant activities. These compounds have also showed potent anticancer and antibacterial activities. This is concluded from the results of this study that curcumin analogs can be used as a possible treatment for the neurodegenerative diseases, cancers and bacterial infections. Further studies are needed to explore their pharmacological profile for other ailments.

CONCLUSION

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

HH wrote the manuscript. SU, NA, andSWA designed the ideas. AU, AS, SUR, MG, ZK,and HH, SU, ZI reviewed the manuscript.

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