Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(10):159-166

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, Characterization and Pharmaceutical Applications of Novel Random Aliphatic Copolythioesters

Kosinapogu Sudhakar* and R Nanthini

Department of Chemistry, Pachaiyapps's College, Chennai, India

---

ABSTRACT

The novel random linear copolythioester Polydecylthiodipropionate-co-decyldodecanedioate (PDTDD), a class of biopolymer including sulfur in the form of a thioester in the polymer backbone was synthesised by mixing 3,3'thiodipropionic acid, dodecanedioic acid and 1,10 decanediol monomers with titanium tetra isopropoxide catalyst using direct melt polycondensation method. The different physical properties of copolyester such as inherent viscosity by Ubbelohde viscometer, solubility test by various solvents, Tg by Differential Scanning Colorimetry (DSC) and crystalline nature by X-ray diffraction (XRD) technique was determined and studied in detail. The chemical structure of the copolyester was investigated by FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The synthesized compounds were tested for human pathogenic bacteria using well diffusion method, in vitro cytotoxicity against normal (Vero cell line) and cancer (A_{549} lung cancer cell line) by MTT assay. Also in vitro antioxidant property of copolymer was studied.

Keywords: Polydecylthiodipropionate-co-decyldodecanedioate; 3,3'-thiodipropionic acid; Dodecanedioic acid; 1,10 decanediol

---

INTRODUCTION

The Polyesters containing sulphur atoms in the main linear chain finds wider application as rubber modifiers and nonvolatile plasticizers. Hydroxyl terminated aliphatic and aromatic thiopolyesters are used as poliol components in the synthesis of high elasticity polyurethane elastomers [1]. Poly (3HB-co-3MP) is the first biopolymer which are designated as polythioesters contains sulfur in the polymer backbone [2]. The only other sulfur-containing biopolymers known are proteins, some complex polysaccharides, and very recently described PHAs allow various applications in medicine, pharmacy, agriculture, packaging and food industry, as active agents or as coatings or carriers [3]. Linear copolymeric polyesters Poly (3,3'-thiodipropionic acid-co-1,6-hexanediol) and poly (3,3'-thiodipropionic acid-co-1,12-dodecanediol) by esterification of an equimolar mixture of 3,3'-thiodipropionic acid and 1,6-hexanediol or 1,12-dodecanediol catalyzed by immobilized lipase B from *Candida antarctica* (Novozym 435) were extracted from the reaction mixtures using tetrahydrofurane and precipitated from tetrahydrofurane-iso-hexane [4]. An exhaustive literature survey revealed that there are only few reports on the synthesis of polythioesters using 3,3'-Thiodipropionic acid. The special monomer 4-thiaheptane-1,7-dioic acid is used as a primary or secondary antioxidant and color stabilizer for polymers including polyolefins, styrenics, rubbers and soap industry. It is also used as an intermediate for the synthesis of many organic compounds. The above mentioned special monomer has wider applications which lead us to extend our area of research towards polythioesters. In this article,

we reported the synthesis of low molecular weight linear copolythioesters by using special monomer 3, 3'-thiodipropionic acid which has various industrial and biological applications.

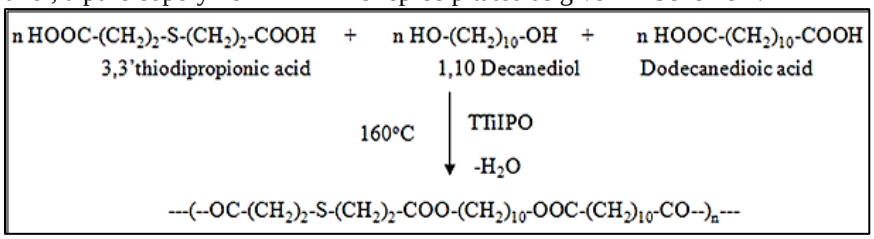
EXPERIMENTAL SECTION

Materials and Methods

Dodecanedioic acid, 3,3'thiodipropionic acid and 1,10 decanediol was purchased from Sigma Aldrich. The catalyst titanium tetra isopropoxide was purchased from Lancaster. All other chemicals and solvents (AR Grade) were purchased from Sigma Aldrich, Mumbai. FT-IR spectra were recorded on Perkin Elmer 883 Spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra for copolyester was recorded on a Bruker 400 MHz and Bruker 100 MHz Spectrometer respectively using $CDCl_3$ as a solvent. DSC thermogram was recorded on DSC Q200 V23.10 Build 79 Differential Scanning Calorimeter. Bruker B8 wide angle XRD with Cu/30 kv/15 mA was used for assessing crystalline or amorphous nature of copolymer. Biological application of PDTDD carried out such as *in vitro* antioxidant activity by Dot Blot assay, *in vitro* antioxidant activity with spectrophotometre at 15.6 µg/mL, 31.2 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL [5] *in vitro* antibacterial activity well diffusion method [6] with 250 µg/mL, 500 µg/mL and 1000 µg/mL against the human pathogens such as *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus* and *in vitro* cytotoxicity of *Vero* (normal) cell line and Lung cancer (A_{549}) line with various concentrations of PDTDD like 7.8 µg/mL, 15.6 µg/mL, 31.2 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL [7].

Synthesis of Copolyester

A mixture of 3,3'thiodipropionic acid (0.01 mole), dodecanedioic acid (0.01 mole) and 1,10 decanediol (0.02 mole) was taken in a three-neck RB flask. One of the left inlet of RB flask connected to nitrogen cylinder, right inlet with stopper and middle inlet with $CaCl_2$ guard tube. The setup is kept in oil bath and heated at its melting point. After the complete melt of mixture 0.8 ml of titanium tetra isopropoxide catalyst is added and kept for one hour. Later the temperature is increased by 25°C and maintained for 2 hrs [8-11]. At half an hour interval the recrystallisation is checked with the reaction sample. The obtained crude copolymer sample is dissolved in chloroform/THF and poured into ice cold methanol, a pure copolymer PDTDD is reprecipitated as given in Scheme 1.



Scheme 1: Synthesis of copolyester PDTDD

RESULTS AND DISCUSSION

Characterization of Physical Parameters

Solubilities of the synthesised copolymer were determined in various solvents. It has been found that the copolymer is soluble in chloroform, dimethyl formamide, THF and DMSO. Inherent viscosity of the copolymer PDTDD is 0.783 and was determined by flow times of solvent and one percent solution of the copolymer dissolved in Chloroform and taken in Ubbelohde viscometer. The DSC thermogram of the colpolymer PDTDD (Figure 1) shows glass transition temperature (T_g) at -50°C and melting temperature (T_m) at 63.48°C which signifies the copolymer has low molecular weight and may be used as plasticizers, stabilizers and very good antioxidants like PBA, PVC etc., Wide XRD of the synthesized copolymer (Figure 2) gives the value of 2θ (deg) = 22-24°C which confirms that the polymer is amorphous in nature [12].

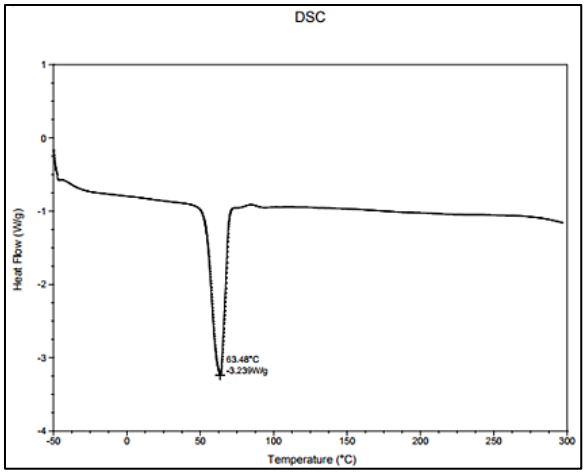


Figure 1: DSC thermogram of the copolymer

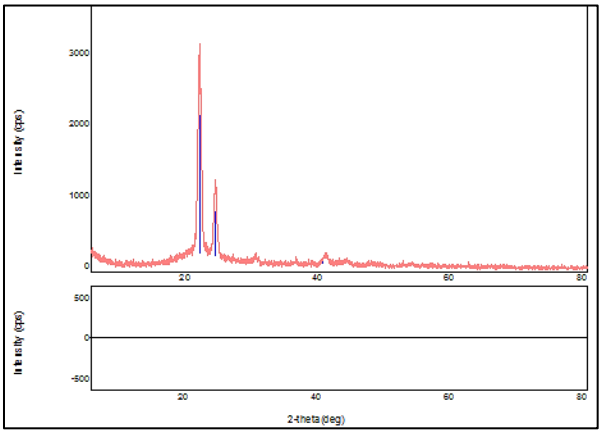


Figure 2: Wide XRD of the copolymer

Structural Elucidation

The FT-IR spectrum (Figure 3) of the synthesized copolyester PDTDD showed characteristic absorption band for ester carbonyl stretching at 1731.19 cm^{-1}. Also the polymer was observed peaks at 648.11, 1233.53, 2929.07 and 1458.25 cm^{-1} due to C-S stretching, C-O-C asymmetric stretching, aliphatic C-H stretching of methylene group and aliphatic C-C stretching respectively [13,14]. A new ester bond that was formed during polycondensation can be revealed from the report. ^{1}H-NMR spectrum (Figure 4) and ^{13}*C*-NMR spectrum (Figure 5) of the copolyester was recorded at RT in $CDCl_3$ solvent. Based on ^{1}H-NMR and ^{13}C-NMR spectral data, it may be concluded that the structural units (Tables 1 and 2) are randomly distributed in the copolyester.

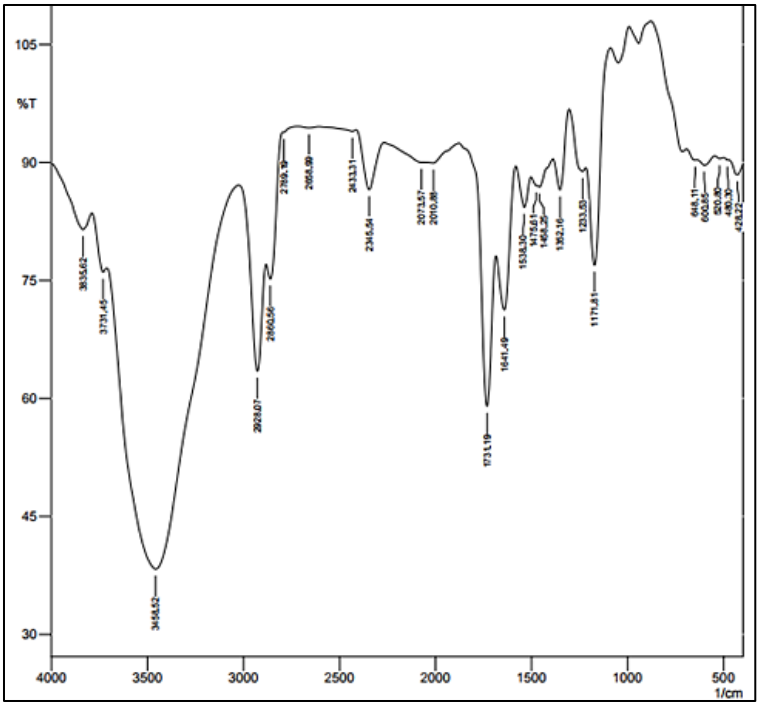


Figure 3: FT-IR spectrum of the copolyester

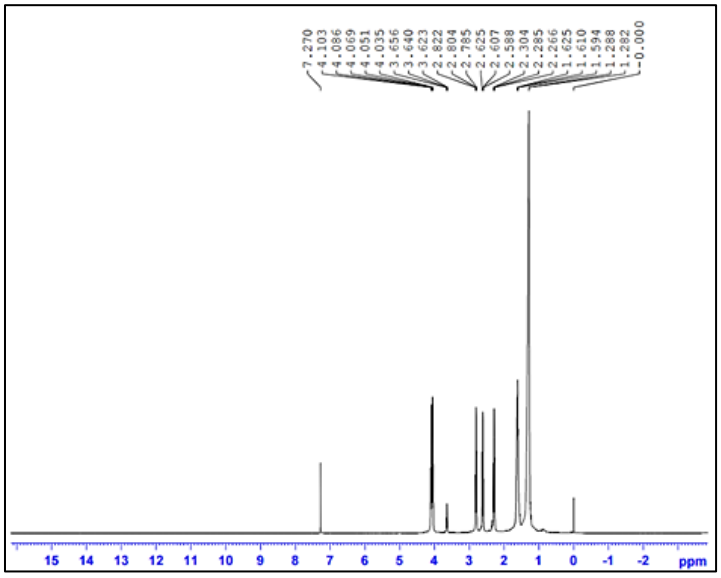


Figure 4: ^{1}H-NMR spectrum of the copolyester

Table 1: Chemicl shift of protons

Chemical Shift in ppm	Type of Proton
1.282 – 1.288	-CH_2- protons
1.594-1.625	-CH_2-S- protons
2.266 – 2.822	-CH_2-CO- protons
3.623 - 3.656	-CH_2-O- protons
4.035 – 4.103	Free -OH (unreacted alcohol)
7.27	$CDCl_3$Solvent

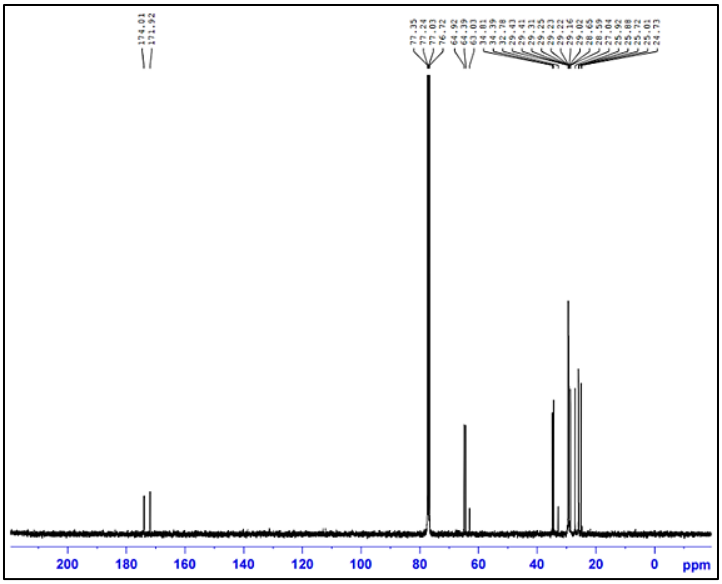


Figure 5: ^{13}C-NMR spectrum of the copolyester

Table 2: Chemicl shift and carbon environment

Chemical Shift in ppm	Carbon Environment
24.73 - 25.92	-CH_2-
27.04	-CH_2-S-
28.59 - 34.81	-CH_2-CO-
64.39 - 77.35	-O-CH_2-
171.92 & 174.01	-C=O (ester group)

Biological Studies

Synthesized compounds were screened for cytotoxicity on *Vero* (normal) cell line and Lung cancer (A_{549}) line with different concentrations of PDTDD like 7.8 µg/mL, 15.6 µg/mL, 31.2 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL (Tables 3 and 4). The compound showed different ranges of viability, cell shrinkage in dose dependent manner (Figures 6 and 7), observed under stereomicroscope. At the maximum concentration of PDTDD (1000 µg/mL), the cell viability were 52.45% for Vero cell line and 10.17% for lung cancer A_{549} cell line. PDTDD showed more toxic on lung cancer cell than normal Vero cell line. Fifty percent death (IC50) was calculated at the concentration of 953.28 µg/mL Vero cell and 31.33 µg/mL lung cancer cell. *In vitro* antioxidant property of PDTDD copolymer showed radical scavenging activity on thin layer chromatography purple color of DPPH radical turned into yellow which confirms the polymer has antioxidant activity by Dot-Blot assay (Tables 5 and 6). Fifty percent radical scavenging activity also studied at the concentration of PDTDD (241.40 µg/mL). *In vitro* antimicrobial activities ranges exhibited 10 to 15 mm and inhibition percentage 11.22 to 16.67% against the pathogens (Figure 8 and Table 7) by well diffusion method.

Table 3: *In vitro* cytotoxicity effect of synthesized compound PDTDD on Vero (normal) cell line

Concentration of compounds (µg/ml)	Cell viability (%)
1000	52.45
500	57.43
250	64.22
125	70
62.5	76.79
31.2	83.05
15.6	88.23
7.8	93.74
Cell control	100
IC 50 value(µg/mL)	953.28

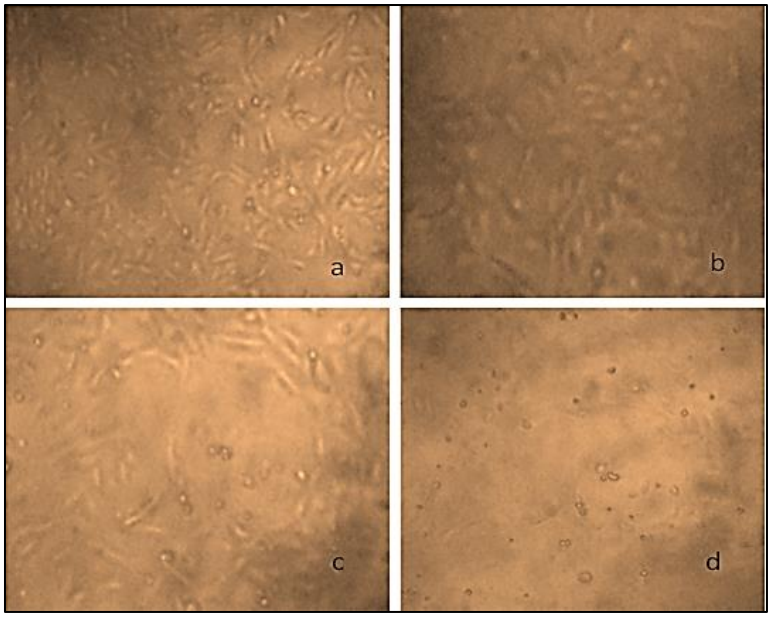


Figure 6: *In vitro* cytotoxicity of synthesized compound PDTDD on normal *vero* cell line, a) Normal cell, b) 7.8 µg/ml, c) 125 µg/ml, d) 1000 µg/ml

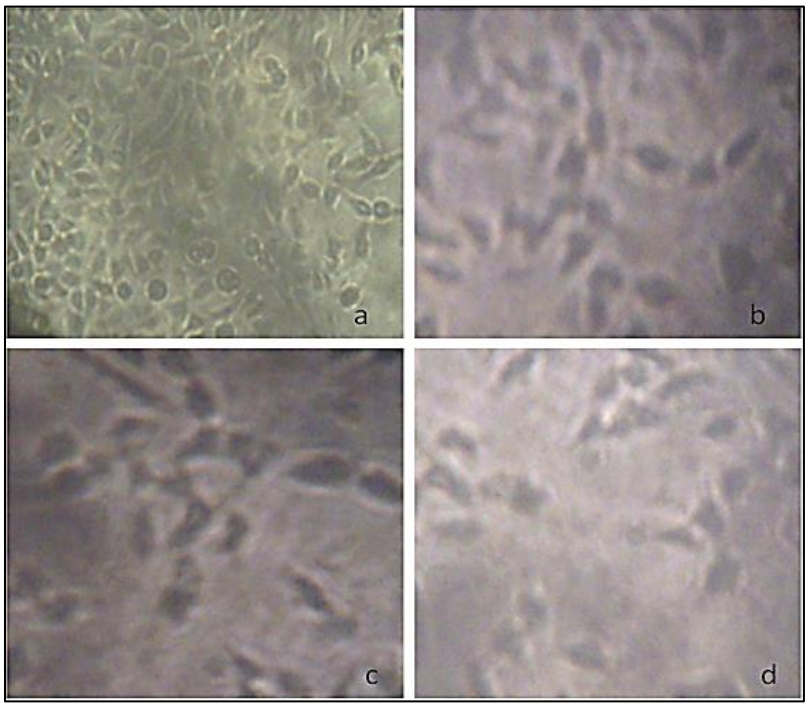


Figure 7: *In vitro* cytotoxicity of synthesized compound PDTDD on lung cancer (A549) cell line, a) Normal cell, b) 7.8 µg/ml, c) 125 µg/ml, d) 1000 µg/ml

Table 4: *In vitro* cytotoxicity of synthesized compounds PDTDD against lung cancer (A549) cell line

Concentration of compounds (µg/ml)	Cell viability (%)
1000	10.17
500	18.67
250	27
125	34.02
62.5	40.57
31.2	49.79
15.6	61.39
7.8	73.4
Cell control	100
IC 50 value(µg/mL)	31.33

Table 5*: In vitro* DPPH activities of synthesized compound PDTDD

Conc. Of Compounds (µg/mL)	% inhibition PDTDD
1000	70.62 ± 4.94
500	75.51 ± 5.28
250	51.78 ± 3.62
125	32.78 ± 2.29
62.5	27.59 ± 1.93
31.25	16.46 ± 1.15
15.62	14.09 ± 0.98
IC_{50} (µg/mL)	241.4

All the values are mean values of Triplicates (Mean ± Standard Deviation)

Table 6*: In vitro* DPPH antioxidant activities of standard (Quercetin)

Conc. of Compounds (µg/mL)	% of Inhibition
1	7.58 ± 0.53
2	15.17 ± 1.06
3	22.75 ± 1.59
4	30.34 ± 2.12
5	37.92 ± 2.65
6	45.51 ± 3.18
7	53.09 ± 3.71
8	60.68 ± 4.24
10	75.85 ± 5.30
IC_{50} (µg/mL)	6.59

All the values are mean values of Triplicates (Mean ±Standard Deviation)

Table 7: Antimicrobial activity of synthesized compound PDTDD against human pathogens by well diffusion method

Human Pathogens	Concentration (in µg)	Zone of inhibition in mm (Percentage of Inhibition)	
		PDTDD	Kannamycin (30 µg)
Escherichia coli	1000	12 ± 0.84 (13.33 ± 0.93)	26.33 ± 1.52 (29.25 ± 1.38)
	500	11 ± 0.77 (12.22 ± 0.85)	
	250	10 ± 0.7 (11.11 ± 0.78)	
Klebsiella pneumoniae	1000	12 ± 0.84 (13.33 ± 0.93)	30.67 ± 1.52 (34.07 ± 1.38)
	500	10 ± 0.7 (11.11 ± 0.77)	
	250	-	
Bacillus subtilis	1000	13 ± 0.91 (14.44 ± 1.01)	27.00 ± 1.00 (30.00 ± 0.90)

	500	11 ± 0.77 (12.22 ± 0.85)	
	250	10 ± 0.7 (11.11 ± 0.78)	
Staphylococcus aureus	1000	12 ± 0.84 (13.33 ± 0.93)	26.00 ± 1.00 (29.25 ± 0.52)
	500	10 ± 0.77 (11.11 ± 0.78)	
	250	-	

All the values are mean values of triplicates (Mean ±Standard Deviation) (-) = Activity Absent

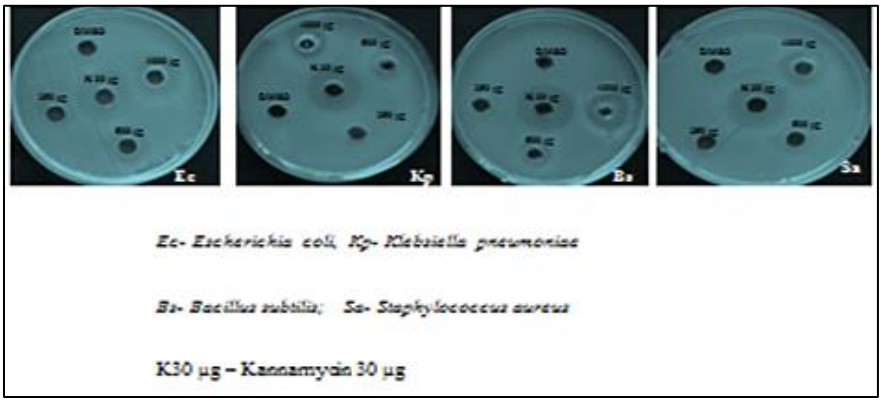


Figure 8: Antimicrobial activity of synthesized compound PDTDD against human pathogens by well diffusion method

ACKNOWLEDGEMENT

The authors grateful to the Principal and Head of Department, Pachaiyappa's college, Chennai for laboratory facility, Central Leather Research Institute (CLRI) and VIT-Vellore for their analytical support and East West Integrative Medicine Center, Chennai for their biological study.

REFERENCES

[1] MF Herman. Encylopedia of Polymer Science and Technology concise, 3rd Edition, John Wiley & sons, 1169, **2013**.
[2] LE Tina; B Klaus; L Heinrich; S Alexander. *Microbiol*. 147, **2001**, 11-19.
[3] S Alexander; LE Tina; E Christian. *Patent US*. 6495152 B2, **2000**.
[4] E Fehling; K Vosmann; K Bergander; N Weber. *Biotechnol Bioeng*. **2008,** 99(5), 1074-1084.
[5] MS Blois. *Nature*. **1958**, 181, 1199-1200.
[6] C Perez; M Pauli; P Bazerque. *Acta Biol Med Exp*. **1990**, 15, 113-115.
[7] T Mosmann. *J Immunol Methods*. **1983**, 65, 55-63.
[8] M Motonobu; N Yutaka; H Tohru. *Patent US*, 4321191, **1982**, 1-38.
[9] B Alan; JK Charles; GS James; Kingsport. *Meth Glycol*. **1962**, 215, 768.
[10] JK Charles; E Clarence. *Patent US*. 3157517, **1964**.
[11] C Liu; JB Zeng; SL Li; YS He; YZ Wang. *Polymer*. **2012,** 53, 481-489.
[12] J Du; Y Zheng; J Chang; L Xu. *European Polymer J*. **2007**, 43, 1969-1977.
[13] MJ Margaret; R Puvanakrishnan; R Nanthini. *J Chem Pharm Res*. **2012**, 4, 175-179.
[14] J Gowsika; R Nanthini. *Hindawi Publishing Corporation J Chem*. **2014**, 173814, 7.