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A STUDY ON THE SYNTHESIS AND BIOCIDAL EFFICACY OF CERTAIN RANDOM COPOLYESTERS CONTAINING CHALCONE MOIETY

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ABSTRACT

A series of four random copolyesters were synthesized by the polycondensation of a chalcone diol by making use of two diacid chlorides namely terephthaloyl chloride and succinyl chloride. The four chalcone diols involved are (*2E*)-1,3-bis(4-hydroxyphenyl)-prop-2-en-1-one, (*2E*)-1-(4-hydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one, (*2E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one and (*2E*)-1,3-bis(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one were synthesized by acid catalyzed Claisen-Schmidt reaction. These copolyesters were characterized by qualitative solubility tests and viscosity measurements. The microstructure of the repeating units available in the copolyester main chain was established by FT-IR, ^{1}H-NMR and ^{13}C-NMR analytical techniques. The inhibitory activity of these random copolyesters against certain bacteria and fungi is well documented. Hence, these random copolyesters may emerge as antimicrobial agents.

KEY WORDS: **Chalcone, Random copolyesters, Polycondensation, Bactericidal, Fungicidal.**

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INTRODUCTION

Chalcones are the aromatic compounds having α,β-unsaturated ketones which turn out to be the central core for a range of significant biological compounds and exist in nature as precursors of flavonoids and isoflavonoids. Recent investigation indicates that chalcones have been one of the major research subject for their interesting biological activities[1,2,3]. Innumerable biological activities are associated with them as they are found to be antitumor[4,5], antioxidant[6,7], antimalarial[8,9], anti-inflammatory[10], antiulcerative[11], antiplatelet[12] and analgesic[13] in nature. The inhibitory behaviour of chalcones against pathogenic bacteria is also well documented in the literature. Sivakumar et al.[14] done the effective antibacterial adhesive coating on cotton fabric using ZnO nanorods and chalcones and studied their antimicrobial activity. Chitra et al.[15] synthesized copolyesters containing bis(chalcone) moiety in the main chain and found that these polymers exhibited significant bacterial activity against pathogenic bacteria. Hsieh et al.[16] reported the anti-inflammatory effect of chalcone derivatives. Dimmock et al.[17] reported the use of bis(benzylidene) cycloalkanone as a potential cytotoxic analogue against human cancer cells. In addition to their utility in the pharmaceutical domain, they also find wide applications in dyes[18] (Asiri, 2003) and cosmetic compositions[19] (Forestier et al., 1989) and also show non-linear optical properties with excellent blue light transmittance[20] (Shettigar et al., 2006). Antibacterial polymers generally have biocidal pendant groups or biocidal repeat units in the polymer chain which has the ability to inhibit the growth of microorganisms such as bacteria, fungi or protozoans. In recent years, antimicrobial materials have a wide range of applications such as medical implants, surgical equipments and protective apparels in hospitals, biosensors, food storage, water treatment, agriculture, marine and industrial equipments. Hence, there is an acute need to develop polymeric materials having antimicrobial activity The objective of the present investigation is to generate four copolyesters by incorporating the chalcone moieties in the main chain by polycondensation, then characterizing them by utilizing appropriate analytical techniques and finally studying their anti-microbial activities. Copolyesters[21] are a class of macromolecules which contain ester linkages and are synthesized by the copolymerization of a diol with a diacid chloride-I and diacid chloride-II in the mole ratio of 2:1:1.

MATERIALS AND METHODS

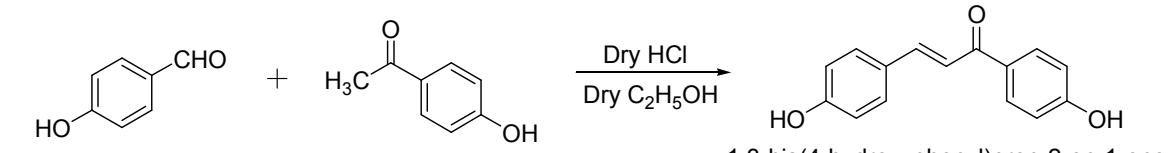
Aldrich samples of 4-hydroxybenzaldehyde, vanillin, 4-hydroxy-3-methoxyacetophenone and 4-hydroxyacetophenone were used as received. Ethanol (Merck) was used as non-solvent for the copolyesters and as solvent for the preparation of the four chalcone diols. Aldrich samples of terephthaloyl chloride and succinyl chloride were purchased and involved as such in the synthesis of the four copolyesters. Spectral grade DMSO-d6 (Aldrich) containing as internal standard was used for recording NMR Spectra.

Synthesis of Chalcone Diols

The monomer diols namely (*2E*)-1,3-bis(4-hydroxyphenyl)-prop-2-en-1-one (BHPP), (*2E*)-1-(4-hydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (HMPP), (*2E*)-1,3-bis(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (BHMP) and (*2E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxy phenyl)-prop-2-en-1-one (HMHP) were synthesized by the process reported by Sidharthan and coworkers[22].

Preparation of BHPP

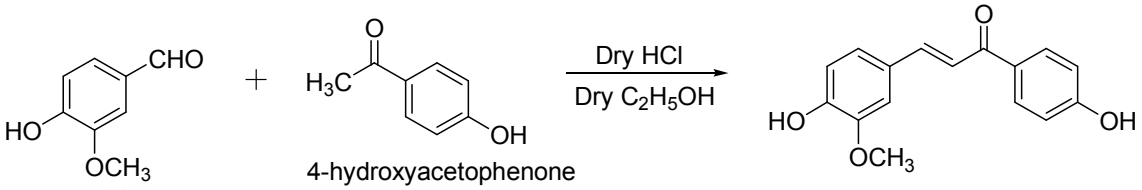
Dry HCl gas was passed through a well-cooled and stirred solution of 4-hydroxy benzaldehyde (50mmol) and 4-hydroxy acetophenone (50mmol) in 100mL of dry ethanol taken in a 250mL round-bottomed flask. The yellow coloured crystals of BHPP which got separated was washed with double distilled water and re-crystallized from hot methanol. Yield: 90% m.p.:183.2°C; IR(KBr) 3301 (b, O–H), 1648(s, C=O) cm^{-1}; ^1H NMR (DMSO-d6) δ 9.9 (s, 2H, –OH), δ 7.4–8.4 (m, 8H, aromatic), δ 6.7–6.9 (dd, 2H, –CH=CH–) and; MS (EI) m/z 240 [M]+.



4-hydroxybenzaldehyde 4-hydroxyacetophenone 1,3-bis(4-hydroxyphenyl)prop-2-en-1-one

Preparation of HMPP

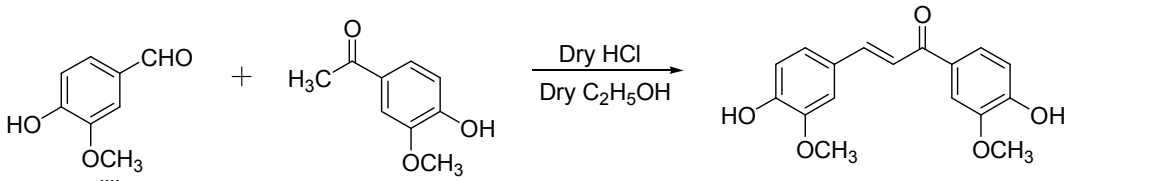
Dry HCl gas was passed through a well-cooled and stirred solution vanillin (50mmol) and 4-hydroxyacetophenone (50mmol) in 100mL of dry ethanol taken in a 250mL round-bottomed flask. The yellow coloured crystals of HMPP which got separated was washed with double distilled water and recrystallized from hot methanol. Yield: 85% m.p.:202°C; IR(KBr) 3320 (b, O–H), 1658(s, C=O) cm^{-1}; ^1H NMR (DMSO-d6) δ 10.1 (s, H, –OH), δ 9.0 (s, H, –OH), δ 7.2–8.3 (m, 7H, aromatic), δ 6.7–6.9 (dd, 2H, –CH=CH–) δ and; MS (EI) m/z 270 [M]+.



vanillin 4-hydroxyacetophenone 3-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

Preparation of BHMP

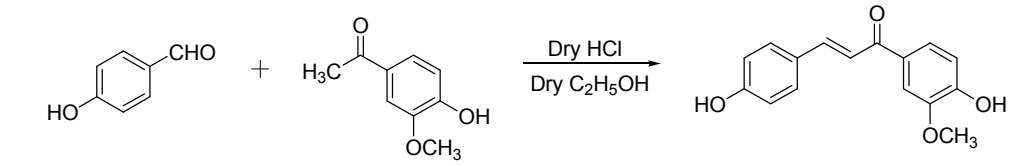
Dry HCl gas was passed through a well-cooled and stirred solution of vanillin (50mmol) and 4-hydroxy-3-methoxy acetophenone (50mmol) in 100mL of dry ethanol taken in a 250mL round-bottomed flask. The yellow coloured crystals of BHPP which got separated was washed with double distilled water and re-crystallized from hot methanol. Yield: 83% m.p.:110°C; IR(KBr) 3320 (b, O–H), 1658(s, C=O) cm^{-1}; ^1H NMR (DMSO-d6) δ 9.9 (s, 2H, –OH), δ 7.2–8.3 (m, 6H, aromatic), δ 6.7–6.9 (dd, 2H, –CH=CH–) δ and; MS (EI) m/z 330 [M]+.



vanillin 4-hydroxy-3-methoxyyacetophenone 1,3-bis(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one

Preparation of HMHP

Dry HCl gas was passed through a well-cooled and stirred solution of 4-hydroxy benzaldehyde (50mmol) and 4-hydroxy-3-methoxyacetophenone (50mmol) in 100mL of dry ethanol taken in a 250 mL round-bottomed flask. The yellow coloured crystals of HMHP which got separated was washed with double distilled water and re-crystallized from hot methanol. Yield: 86% m.p.:182°C; IR(KBr) 3320 (b, O–H), 1658(s, C=O) cm^{-1}; ^1H NMR (DMSO-d6) δ 10.1 (s, H, –OH), δ 9.2 (s, H, –OH), δ 7.2–8.3 (m, 7H, aromatic), δ 6.7–6.9 (dd, 2H, –CH=CH–) δ and; MS (EI) m/z 270 [M]+.



4-hydroxybenzaldehyde 4-hydroxy-3-methoxyyacetophenone

(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one

SYNTHESIS OF COPOLYESTERS

The procedure[23] involved in the preparation of an aliphatic diacid chloride-based copolyester is presented here. The monomer BHPP (0.8g) was dissolved in 10ml of DMF and taken in a round bottomed flask (100ml). After 5 minutes 1ml of triethylamine was added to the reaction mixture and it was stirred well in an inert atmosphere for about 15 minutes. Then, about 0.2ml solution of terephthaloyl chloride and 0.2ml of succinyl chloride were added with in a period of 10 minutes with constant stirring after which the temperature was raised to 100°C and maintained at this temperature with continuous stirring for a span of about 3 hours. Finally the reaction mixture was cooled to room temperature and poured into 100ml of methanol when the copolyester was precipitated. It was filtered, washed with dry methanol and dried in a vacuum. The other three copolyesters PBTS, PCTS and PDTS were also prepared by a similar method.

Table 1
Monomers used, Copolyester Code and their respective % Yield and Inherent Viscosities

Diol	Diacid Chloride - I	Diacid Chloride - II	Copolyester Code	Yield (%)	η_{inh}, (dL/g)
BHPP	Terephthaloyl chloride	Succinyl chloride	PATS	76	0.73
HMPP	Terephthaloyl chloride	Succinyl chloride	PBTS	71	0.84
HMHP	Terephthaloyl chloride	Succinyl chloride	PCTS	77	0.96
BHMP	Terephthaloyl chloride	Succinyl chloride	PDTS	74	1.10

ANTIMICROBIAL ACTIVITY (AGAR DISC DIFFUSION METHOD)

Preparation of Inoculums

Stock cultures were maintained at 4°C on the slant of nutrient agar. Active cultures for experiments were prepared by transferring of a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hours at 37°C. The assay was performed by the agar disc diffusion method.

Antibacterial Activity

The disc diffusion method[1] was employed to establish the antibacterial activity. The antibacterial activity of polymer sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The MHA medium was weighed as 3.8gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media were poured in to sterile Petri plates, these Petri plates were allowed to solidify for twenty minutes. After the medium was solidified, the inoculums were spread on the solid plates with a sterile swab moistened with the bacterial suspension. The discs were placed on MHA plate and 20µl of sample [concentration: 1000µg, 500µg, 250µg, 125µg, 62.5µg] were added. The plates were incubated for 24 hours, at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition[24].

Antifungal Activity

Antifungal activity of polymer sample was determined by antifungal susceptibility test. Prepare potato dextrose agar (PDA) broth and inoculate the culture. Then it is kept in shaker for a day. The PDA was weighed as 3.9gms and dissolved in 100mL of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media were

poured in to sterile Petri plates, these Petri plates were allowed to solidify for twenty minutes. After solidification, the inoculums were spread on the solid plates with a sterile swab moistened with the fungal suspension. The discs were placed in PDA plate and 20µl of sample [concentration: 1000µg, 500µg, 250µg, 125µg, 62.5µg] were added. The plates were kept at room temperature. Then the microbial growth was determined by measuring the diameter of the zone of inhibition.

RESULTS AND DISCUSSION

The copolyesters synthesized in the present investigation were characterized by solubility studies in various solvents qualitatively and viscosity measurements at a concentration 0.1gdL^{-1} using Ubbelohde viscometer at 30°C. The FT-IR spectra of the four copolyesters were recorded by means of Shimadzu FT-IR instrument and that of ^{1}H and ^{13}C-NMR spectra were obtained involving BRUKER AV III 500 MHz instrument in DMSO-d6 solvent. Disc diffusion method was employed to assess the anti-bacterial and fungicidal activity of the copolyesters.

Solubility

The copolyesters presented here are seen to be soluble in highly polar solvents like N,N-dimethyl acetamide (DMAc) and N,N-dimethyl formamide, partially soluble in moderately polar solvents like acetone and tetrahydrofuran and thoroughly insoluble in least polar solvents like benzene and hexane. Copolyesters with methoxy substituent in the benzene ring of the chalcone moiety had greater solubility than those without methoxy substituents which may be to their aptitude to disrupt the macromolecular chain. Parallel remarks were made by Chitra and coworkers[25] in a series of copolyester.

Viscosity Measurements

The η_{inh} value of all the four copolyesters was determined in DMAc solution at 30ºC using Ubbelohde viscometer. In each case about 25mg of the pure dry copolyester sample was dissolved in 25ml of DMAc, kept aside for some time with occasional shaking. The η_{inh} was calculated from the flow time measurements by using the formula $\eta_{inh} = \ln(t/t_0)/C$, where C is the concentration of the polymer solution. The inherent viscosity values were found to be in the range of dL/g and are presented in table 1. The data infers that these copolyesters are of reasonably high molecular weight.

Spectral Studies

The FT-IR Spectra of the four copolyesters were recorded using Shimadzu FT-IR instrument and they showed characteristic absorption in the range of 1742-1764cm^{-1} which is as a result of the ester carbonyl carbon-oxygen stretching frequency. Similar observations were made by Samuel et al[26] in a series of copolyesters. The ^{1}H-NMR and the ^{13}C-NMR spectra were recorded with BRUKER AV III 500MHz instrument in DMSO-d6 solvent to identify the structural units existing in the copolyester chain. The aromatic protons were observed in the range of 7.5-8.0ppm. The vinylic protons attached to the carbonyl carbon were observed in the range of 6.7-7.0ppm. The methoxy protons in the chalcone moiety are shown by a signal at 3.4ppm. The methylene protons were observed in the range of 2.0-3.0ppm. Similar observations were by Chitra and coworkers[27] in a series of copolyesters derived from chalcone diols. The signals in the range of 170–205ppm and 165–175ppm in the ^{13}C-NMR spectra of the copolyesters are as a result of the α,β-unsaturated ketone and carbonyl carbon of the ester groups, respectively which indicates the formation of copolyester.

Bactericidal Study

The antibacterial activity of the four copolyesters PATS, PBTS, PCTS and PDTS were assayed against *Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus,* and *Escherichia coli* by disc diffusion method[28,29].

Table 2
Inhibition effects of the copolyesters on the growth of S. aureus E. faecalis, B. cereus and E. coli.

Test Material	*Staphylococcus aureus* Zone of inhibition in mm					*Enterococcus faecalis* Zone of inhibition in mm				
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	1000 µg	500 µg	250 µg	125 µg	62.5 µg
PATS	12	8	9	8	-	13	10	8	8	-
PBTS	16	14	13	10	8	18	16	11	9	7
PCTS	15	14	11	9	9	14	13	11	8	8
PDTS	16	14	10	7	-	15	10	9	8	7
Test Material	***Bacillus cereus* Zone of inhibition in mm**					***Escherichia coli* Zone of inhibition in mm**				
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	1000 µg	500 µg	250 µg	125 µg	62.5 µg
PATS	15	11	10	6	6	17	11	11	7	-
PBTS	17	17	12	10	-	20	18	15	12	10
PCTS	15	10	10	9	8	19	16	13	11	10
PDTS	16	12	10	7	6	16	15	14	9	8

Streptomycin inhibited the development of *S. aureus* by 20mm, *E. faecalis* by 19mm, *B. cereus* by 26mm, and *E. coli.* by 17mm. From table 2 it is abundantly conspicuous that the four copolysters were found to be bactericidal in character. With the increase of concentration of the copolyester material it was observed that the inhibition effect increased notably. Similar comments were made by Kannappan et al.[30] in a set of poly(ester-amides) and Vinay Suvarna et al.[31] in Artocarpus hirsutus edible fruit part.

Fungicidal Study

The antifungal activity of the synthesized copolyesters PATS, PBTS, PCTS and PDTS were assayed against *Candida albicans*, A*spergillus flavus, Penicillium and T. mentogrophyte.* by disc diffusion method.

Table 3
Inhibition effects of the copolyesters on the growth of C. albicans, A. flavus, Penicillium and T. mentogrophyte

Test Material	*Candida albicans* Zone of inhibition in mm					A*spergillus flavus* Zone of inhibition in mm				
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	1000 µg	500 µg	250 µg	125 µg	62.5 µg
PATS	8	9	10	10	12	1	1	1	1	1
PBTS	8	8	9	8	7	-	-	-	-	-
PCTS	9	8	8	8	8	7	7	7	7	7
PDTS	6	8	8	8	8	8	8	8	8	8
Test Material	***Penicillium* Species Zone of inhibition in mm**					***T. mentogrophyte* Zone of inhibition in mm**				
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	1000 µg	500 µg	250 µg	125 µg	62.5 µg
PATS	6	7	7	7	7	10	9	9	9	7
PBTS	6	7	7	8	8	9	8	6	6	-
PCTS	10	9	9	9	7	11	10	9	8	6
PDTS	10	9	9	9	9	9	8	-	-	-

Streptomycin suppressed the growth of *C. albicans* by 14mm, A*. flavus* by 13mm, *Penicillium* by 10mm, and *T. mentogrophyte* by 10mm. From table 3 it is obvious that the four copolyesters were found to be fungicidal in activity. Similar observations were made by Jasmine Francis et al.[32] in the copolyesters and Sukanya et al.[33] in Phyllanthus Emblica, a medicinally valued plant.

CONCLUSION

The four copolyesters were synthesized using a diacid chloride-I (terephthaloyl chloride), diacid chloride-II (succinyl chloride) and a chalcone diol. The chalcone diols were varied. The four chalcone diols used are (*2E*)-1,3-bis-(4-hydroxyphenyl)-prop-2-en-1-one (BHPP), (*2E*)-1-(4-hydroxy phenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (HMPP),

(*2E*)-1,3-bis-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (BHMP) and (*2E*)-1-(4-hydroxy-3-methoxy phenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one (HMHP). These copolyesters are highly soluble in polar organic solvents. They were characterized by solubility studies, viscosity measurements and spectral data. Disc diffusion method was employed to establish and document the bactericidal and fungicidal activities of these copolyesters.

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