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Research Article

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Antibacterial activity of diazotized resins from renewable resource

S. G. Jebastin Andrews[1] and C. V. Mythili[2*]

[1]Department of Chemistry, V V College of Engineering, Tisyanvilai, Tirunelveli, Tamil Nadu, India
[2]Department of Chemistry, Rani Anna Govt. College for Women, Tirunelveli, Tamil Nadu, India

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ABSTRACT

Cashew Nut Shell Liquid mainly contains phenolic constituents such as anacardic acid, cardanol and cardol. Cardanol contains polymerizable side chain and phenolic group. Cardanol and its various derivatives have found numerous industrial and medicinal applications. This research paper deals with the synthesis and characterization of diazotized resins synthesized from renewable source cardanol. p-chloroaniline and p-nitroaniline have been diazotized and coupled with cardanol to prepare diazotized p-chloroaniline cardanol dye and diazotized p-nitroaniline cardanol dye respectively. Further, both the dyes have been condensed with furfural and followed by urea separately in order to get the desired diazotized resins. The resins have been characterized by Ultra violet-Visible spectroscopy, Fourier Transform-Infrared spectroscopy and ^{1}H-Nuclear Magnetic Resonance spectroscopy techniques. Also antibacterial activity for the diazotized resins have also been investigated and discussed in detail.

Key words: Cardanol, Diazotized resins, FT-IR, ^{1}H-NMR, Antibacterial activity.

INTRODUCTION

Cashew Nut Shell Liquid (CNSL) being cheap can be a substitute for phenol in many applications. The use of CNSL in place of phenol is an excellent example of conservation of a synthetically derived substance and the utilization of a cheap agro by-product. ln modern context, CNSL occupies a position of importance because of its renewable nature [1]. Azo compounds are important structures in the medicinal and pharmaceutical field [2]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticide activity. In the present time, exploration of azo dye as antibacterial and antifungal agents has received considerable attention [3-5].Azo compounds are known for their medicinal importance and are well recognized for their use as antineoplastic [6], antidiabetics [7], antiseptics [8], antibacterial [9,10], antitumor [11].The quaternary nitrogen compounds prepared form the meta-alkylphenols of CNSL possessed unusually high germicidal activity [12]. The shell oil is used as a mosquito larvicide [13,14]. The CNSL oil is a mild purgative and is used in the treatment of hook worm. It is also used for the treatment of cracks on soles of feet, warts, corns and leprous sores. CNSL inhibits enzymes such as prostogl and in synthetase, lipoxygenase and tyrosinase. Anacardic acid is known to exhibit antitumour, antimicrobial and antiacne properties. This communication presents the preparation of diazotized p-chloroaniline cardanol-furfural-urea resins and p-nitroaniline cardanol-furfural-urea resins. They are characterized by FT- IR, ^{1}H-NMR spectroscopic techniques and their antibacterial activities have also been studied.

EXPERIMENTAL SECTION

Cardanol was obtained from M/s Sathya Cashew Chemicals Ltd, Chennai, Sodium nitrite and potassium hydroxide, Methanol, p-chloroaniline, p-nitroaniline furfural and urea were received from Himedia. The chemicals were used as received. Ultraviolet spectral analysis was carried out in a UV-VIS double beam spectrophotometer 220l Systronics. Infrared spectra of the resins were recorded with Shimadzu FT-IR spectrophotometer by KBr pellet method. The ^{1}H-NMR spectra were recorded using Bruker Advance III 400 MHz FT- NMR spectrometer using D_6- DMSO as a solvent.

RESULTS AND DISCUSSION

Synthesis of diazotized resins

2.2 g of p-chloroaniline was dissolved in 5ml Con.HCl and 5ml hot distilled water.1.2 g of sodium nitrite solution was added at 0°C with constant stirring. 5g of cardanol was dissolved in a chilled solution of alcoholic potassium hydroxide and was added drop wise to the diazonium salt solution. The whole system was kept in ice bath within the temperature range of 0-10°C. The red dye formed was stirred for a period of 6 hours and poured in dil.HCl with constant stirring. The red dye was separated, washed thoroughly with water and dried. The diazotized p-chloroaniline cardanol dye (DC) was recrystallized from methanol water mixture. Then 2.15 g of DC, 3ml furfural, 0.54 g urea and 2 ml 3N. H_2S0_4 were heated at 100 °C in a round bottom flask fitted with condenser at constant stirring using mechanical stirrer for 6 hours. The diazotized *p*-chloroaniline cardanol-furfural-urea resin (DCFR) was washed with dil. NaOH and then with hot distilled water and then dried in vacuum. The yield was 80%. Similarly *p*-nitroaniline (2.43g) was diazotized and coupled with cardanol, furfural and urea to synthesize diazotised p-nitroaniline cardanol-furfural-urea resin (DNFR).

Characterization of diazotised resins

A broad peak centered at 3363 cm^{-1} was appeared in the spectrum (fig.1a) confirmed the presence of phenolic hydroxyl group in the resinous product. The peak that appeared near 2921 cm^{-1} might be due to the presence of aliphatic C-H stretching present in side chain of cardanol. The peak at 1451 cm^{-1} shows the presence of azo group. The sharp band near 2857 cm^{-1} might be due to the C-H stretching in a methylene bridge which might form due to the condensation reaction between cardanol and furfural. A peak at 1586 cm^{-1} is ascribed to N-H bending as well as C=C stretching in aromatic ring. The peak near 1151 cm^{-1} indicates the C-O stretching in ester or alcohol group. The peak at 874 cm^{-1} indicates that the trans double bond in the side chain of cardanol moiety. A sharp intense peak at 727 cm^{-1} indicates the C-H out of plane bending vibration in mono substituted aromatic ring. In the ^{1}H-NMR spectra (Fig.1b) of DCFR, the peak at 6.7-6.8 ppm shows the aryl protons of benzene nuclei and the peak at 5.0 ppm shows the phenolic hydroxyl group. The peak at 7.0-7.5 ppm shows the aryl protons of p-chloroaniline. The peak at 1.3-2.5 ppm shows the methylene group of the long alkyl side chain of cardanol. The peaks at 0.8-2.8 ppm correspond to the aliphatic side chain whereas the peaks at 4.6-5.0 ppm confirmed the presence of unsaturated hydrocarbons in the side chain. In addition to that the C-H proton aroused due to the condensation reaction appeared at 3.7 ppm confirm the formation of expected product.

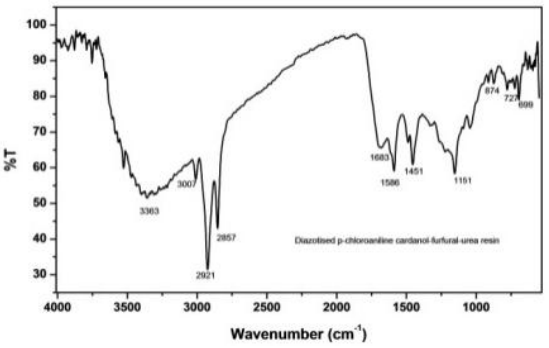

Fig 1a.FT-IR spectrum of DCFR

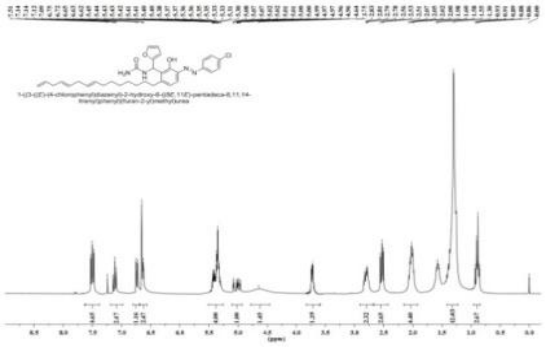

Fig 1b.^{1}H-NMR spectrum of DCFR

In the IR Spectrum (Fig.2a) of DNFR, the peak at 1459 cm^{-1} shows the presence of azo group. The peak at 3404 cm^{-1} shows the O-H stretching frequency of hydrogen bonded phenolic OH group. The peak at 2951cm^{-1} shows the symmetrical CH_2 stretching of the side chain of cardanol. The sharp band near 2871 cm^{-1} might be due to the C-H stretching in a methylene bridge which might form due to the condensation reaction between cardanol and furfural. The peak at 1332 cm^{-1} shows the N=O stretching frequency in presence of aromatic nitro compound. The peak at 862 cm^{-1} shows the C-N stretching frequency in presence of aromatic nitro compound. A peak at 1591 cm^{-1} is ascribed to N-H bending as well as C=C stretching in aromatic ring. In ^{1}H-NMR spectrum of DNFR (Fig.2b), the peaks appeared at 6.6-8.5 ppm correspond to the aryl protons of DNFR and the peak appeared at 5.3 ppm corresponds to the phenolic OH group. The peaks at 0.8-2.8 ppm correspond to the aliphatic side chain whereas the peaks at 4.6-5.5 ppm confirmed the presence of unsaturated hydrocarbons in the side chain. In addition to that the C-H proton aroused due to the condensation reaction appeared at 3.7 ppm confirm the formation of expected product.

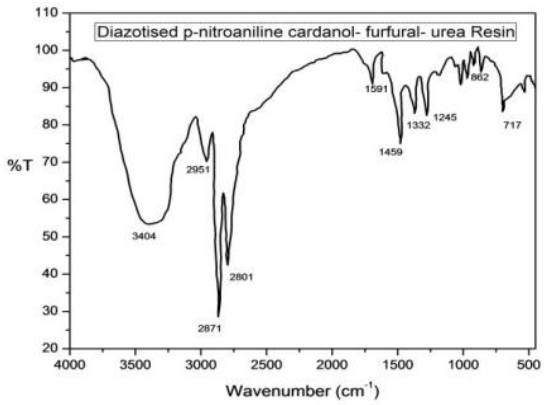

Fig 2a.FT-IR spectrum of DNFR

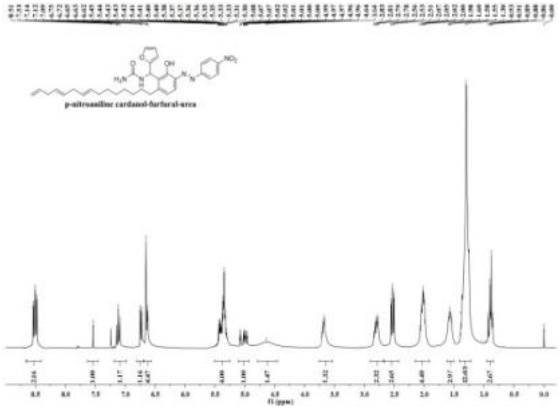

Fig 2b.^{1}H-NMR spectrum of DNFR

Determination of antibacterial assay

Bacterial strains used for testing included E.coli, Staphylococcus aereus and Enterobacter aerogenes. These were obtained from National Chemical Laboratory, Pune, India. The stock culture was maintained on Muller Hinton agar medium (Himedia chemicals) at 37^{o}C. The bacterial culture was incubated for 24 h at 37^{o}C in nutrient agar slants (Himedia, Mumbai, India). Before streaking, each culture was diluted (1:10) with fresh sterile nutrients broth. Plates were prepared by pouring 20 ml of freshly prepared No.1 medium (Himedia, Mumbai, and India) into 20 mm × 100mm Petri plates. Inoculam (5ml) was poured directly over the surface of prepared plates to uniform depth of 4 mm and then allowed to solidify at room temperature. The antibacterial activity was measured by Disc diffusion method [15]. Test drug solution of each diazotized compound was prepared in different concentrations. The test bacterial strains were inoculated into agar plates (Himedia, Mumbai) separately. Then the sterile disc each containing each test solution of the diazotized resins was placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. The plates were kept at room temperature for two hours to allow diffusion of the drug into the agar; they were incubated for 24 h at 37^{o}C for the bacterial strain. After the incubation period was over, the plates were observed for zone of inhibition (ZI) measured in millimeters (mm).

Antibacterial activity of DCFR and DNFR

The bioassay results for antibacterial activity of the DCFR and DNFR are presented in the Table-1 and Table- 2 respectively. The solvent used for the dissolution of the diazotized resins did not show any activity at the volume used. Both DCFR and DNFR exhibited varying degree of antibacterial activity against the test organisms. The DCFR shows the highest antibacterial activity when compared with DNFR.

Table 1. Antibacterial activity of DCFR

Name of the organism	Zone of Inhibition (mm) Concentration of azo compound I					
	5 µg	10 µg	20 µg	30 µg	40 µg	50 µg
Staphylococcus aereus	-	-	2	2.2	1	1
Bascillus cereus	-	-	1	1	1.2	1.2
Enterobacter aerogenes	-	-	-	1	1	1.2
E.coli	-	2.4	7	8	10	12

Table 2.Antibacterial activity of DNFR

Name of the organism	Zone of Inhibition (mm) Concentration of azo compound II					
	5 µg	10 µg	20 µg	30 µg	40 µg	50 µg
Staphylococcus aereus	-	-	-	-	-	2
Bascillus cereus	-	-	-	-	-	1
Enterobacter aerogenes	-	-	-	-	-	1
E.coli	--	-	3	2.8	2.5	-

CONCLUSION

This proposed research may lead to a new platform for antibacterial studies for diazotized cardanol polymers. From the results, it can be concluded that diazotized p-chloroaniline cardanol-furfural-urea resin has significant antibacterial activity than p-nitroaniline cardanol-furfural-urea resin.

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