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**Research Article**

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# Antibacterial activity of diazotised dyes synthesized from cardanol

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**ABSTRACT**

*Cashew nut shell liquid (CNSL) and its derivatives are widely used in polymer-based industries, synthesis of chemicals and intermediates including bactericides, insecticides and surface active agents. Commercially available CNSL 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 applications. p -chloro aniline and p-nitro aniline have been diazotised and coupled with cardanol to produce diazotised-p-chloro aniline cardanol dye and diazotised-p-nitro aniline cardanol dye respectively. They have been characterized by, FT- IR and $^{1}$H-NMR spectroscopic techniques and their antibacterial activity has also been studied. Antimicrobial studies revealed that compound was showed significant activity against tested strains.*

**Key words**: cardanol, p-chloro aniline, p-nitro aniline, diazotisation, antibacterial activity.

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## INTRODUCTION

The cashew tree is evergreen. The Cashew nut has a shell of about 1/8 inch thickness, with a soft honeycomb structure inside, containing a dark brown viscous liquid. It is called cashew nut shell liquid (CNSL), which is pericarp fluid of the cashew nut. Synthesis of most azo dyes involves diazotization, followed by coupling with nucleophiles. Over the years, azo compounds constitute one of the largest classes of industrially Synthesized organic compounds, potent in drug. [1]Azo compounds are important structures in the medicinal and pharmaceutical field [2]. Azo compounds are well recognized for their use as antineoplastic [3], antidiabetics [4], antiseptics [5], antibacterial [6, 7], antitumor [8]. In addition, Evans blue and Congo red are azo dyes being studied as HIV inhibitors of viral replications. This effect is believed to be caused by binding of azo dyes to both protease and reverse transcriptase of this virus [9]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticides activities. In the recent times, exploration of azo dye as antimicrobial agents has received considerable attention [10-13]. Cardanol finds many applications in the form of phenol – formaldehyde resin in varnishes, paints and brake linings [14]. Cardol is also active against the filarial parasite of cattle Setaria digitata[15].The cardanol and anacardic acid have antimicrobial activity against Pseudomonas fluorescence [16].In the present study p -chloro aniline and p-nitro aniline have been diazotised and coupled with cardanol to produce diazotised-p-chloro aniline cardanol dye and diazotised-p-nitro aniline cardanol dye respectively. They are characterized by FT- IR, 1H- NMR spectroscopic techniques and their antibacterial activity has also been studied.

## EXPERIMENTAL SECTION

Cardanol was obtained from M/s Sathya Cashew Chemicals Ltd, Chennai, Sodium nitrite and potassium hydroxide was received from Nice Chemicals (Mumbai). Methanol, p -chloro aniline and p-nitro aniline were received from Loba Chemie. The chemicals were used as received. Infrared spectra were taken in a Shimadzu-FT-IR

spectrophotometer by KBr pellet method. 1H-NMR spectra were taken using Bruker Avance III 400 NMR spectrometer using CDCl3 as the solvent.

**Synthesis of azo compound I and azo compound II**

To 2.2 g of p -Chloro Aniline was dissolved in 5ml Con.HCl and 5ml hot distilled water.1.2 g of sodium nitrite solution was added at $0^oC$ 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 $0^o$-$10^oC$. 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 dye (azo compound I) was recrystallized from methanol water mixture. The yield was 85 %. Similarly p-Nitro aniline (2.43g) was diazotised and coupled with cardanol to synthesise diazotised p-nitro aniline cardanol dye (azo compound II).

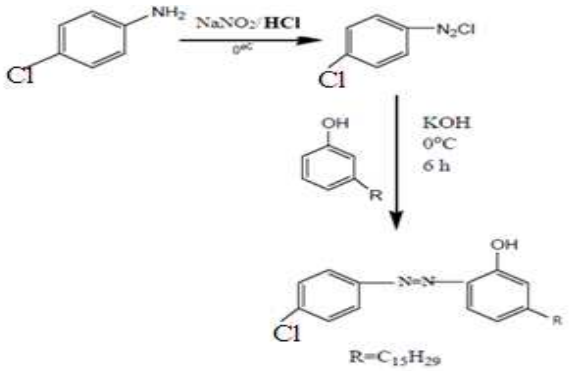
**Determination of antibacterial assay**

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 $37^oC$. The bacterial culture was incubated for 24 h at $37^oC$ 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 × 100 mm 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 [13]. Test drug solution of each azo 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 azo compound 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 $37^oC$ for the bacterial strain. After the incubation period was over, the plates were observed for zone of inhibition (ZI) measured in millimeters (mm).

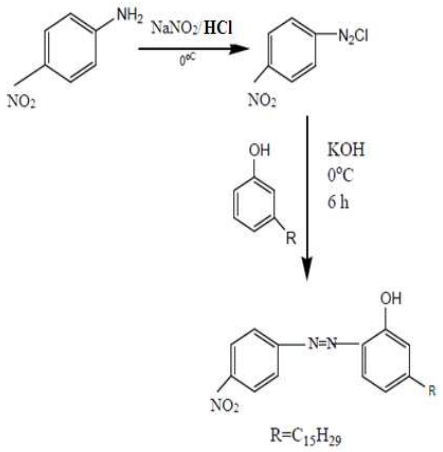
## RESULTS AND DISCUSSION

**Mechanism for the synthesis of azo compound I and azo compound II**

In the first step p-chloro aniline and p-nitro aniline was diazotized in the presence of sodium nitrite and dilute hydrochloric acid catalyst to form diazonium salt. It was then coupled with cardanol to give the azo compound I and azo compound II.




**Scheme 1. Synthesis of diazotised p- Chloro Aniline cardanol dye**




**Scheme 2. Synthesis of diazotised p- Nitro Aniline cardanol dye**

## Characterization

### FT IR and $^1$H-NMR Spectrum of azo compound I

IR Spectral data of diazotised p- Chloroaniline cardanol dye reveal that the diazotization of p- Chloroaniline with cardanol.

In the Fig. 1, FT- IR spectrum of compound I, phenolic hydroxyl group stretching appears at 3404 cm-1. The peak at 2926 cm-1 shows the symmetrical $CH_2$ stretching of the side chain of cardanol. The peak at 1458 cm-1 shows the presence of azo group. In the $^1$H-NMR spectra (Fig.2) of diazotized p- Chloroaniline cardanol dye, 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 7.4-7.8 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 peak at 0.9 ppm shows the terminal methyl group of side chain of cardanol.

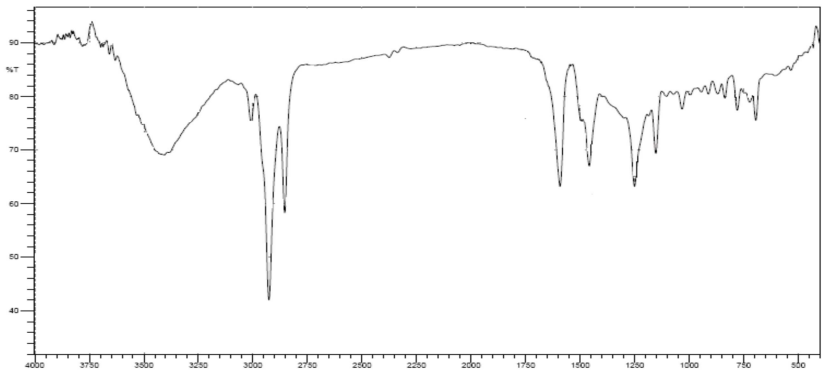



**Fig 1: IR spectrum of p- Chloro Aniline cardanol dye**

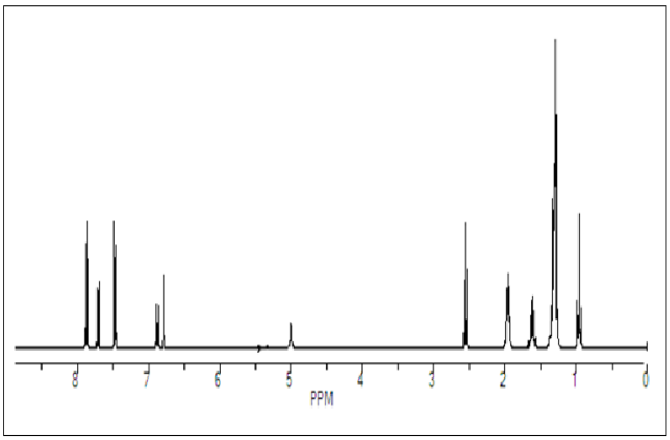



**Fig 2: 1HNMR spectrum of p- Chloro Aniline cardanol dye**

**FT IR and $^1$H-NMR Spectrum of azo compound II**

IR Spectral data (Fig.3) of diazotised p- Nitro Aniline cardanol dye reveal that the diazotization of p- Nitro Aniline with cardanol. The peak at 1458 cm-1 shows the presence of azo group. The peak at 3470 cm-1 shows the O-H stretching frequency of hydrogen bonded phenolic OH group. The peak at 2924 cm-1 shows the symmetrical CH2 stretching of the side chain of cardanol. The peak at 1461 cm-1 shows the presence of azo group. The peak at 1336 cm-1 & 778cm-1 shows the N=O stretching frequency in presence of aromatic nitro compound. The peak at 860cm-1 shows the C-N stretching frequency in presence of aromatic nitro compound. In the NMR spectrum (Fig.4) the peak at 6.8-6.7 ppm shows the aryl protons of cardanol and the peak at 5.2 ppm shows the phenolic hydroxyl group. The peak at 1.9 ppm reveals the presence of long chain containing more than five methylene groups in the side chain. The peak at 8.3- 7.0 ppm shows the aryl protons of p-nitro aniline and the peak at 0.81 ppm show the terminal methyl group of side chain of cardanol.

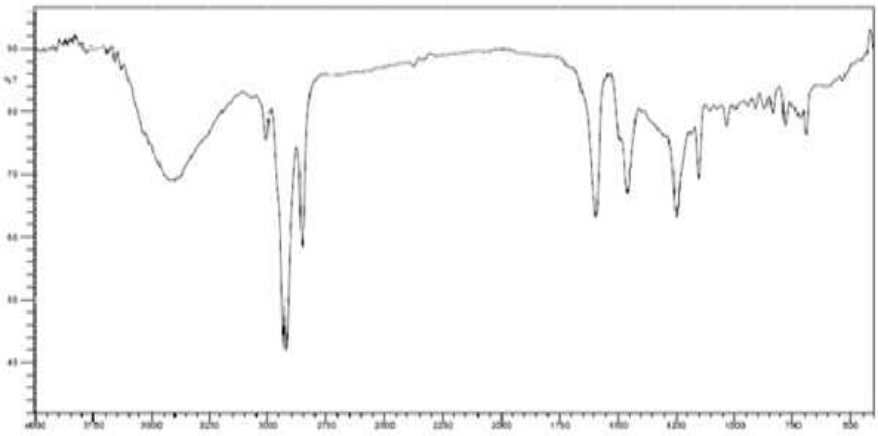


**Fig 3: IR spectrum of p-Nitro Aniline cardanol dye**

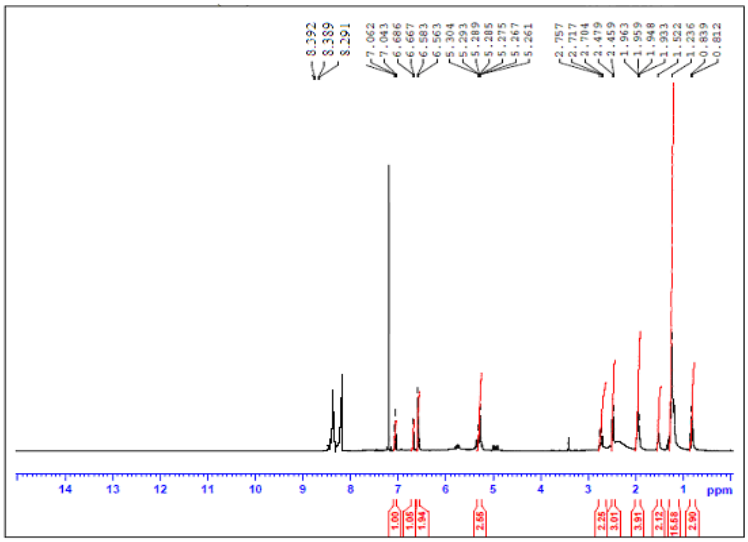



**Fig 4: $^1$HNMR spectrum of p-Nitro Aniline cardanol dye**

**Antibacterial activity of azo compound I & azo compound II**

The bioassay results for antibacterial activity of the azo compounds I and II are presented in the Table1 and Table 2 respectively. The solvent used for the dissolution of the azo compounds did not show any activity at the volume used. Both the azo compound I and azo compound II exhibited varying degree of antibacterial activity against the test organisms. The azo compound I shows the highest antibacterial activity with the inhibition zone of 12 mm against *E.coli*. The azo compound II shows only less antibacterial activity when compared with azo compound I.

**Table 1 Antibacterial activity of azo compound I**

| 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 |
| *E.coli* | - | 3 | 7 | 9 | 10 | 12 |
| *Staphylococcus aereus* | - | - | 2 | - | 1 | 1 |
| *Enterobacter aerogenes* | - | - | - | - | 1 | 1.2 |

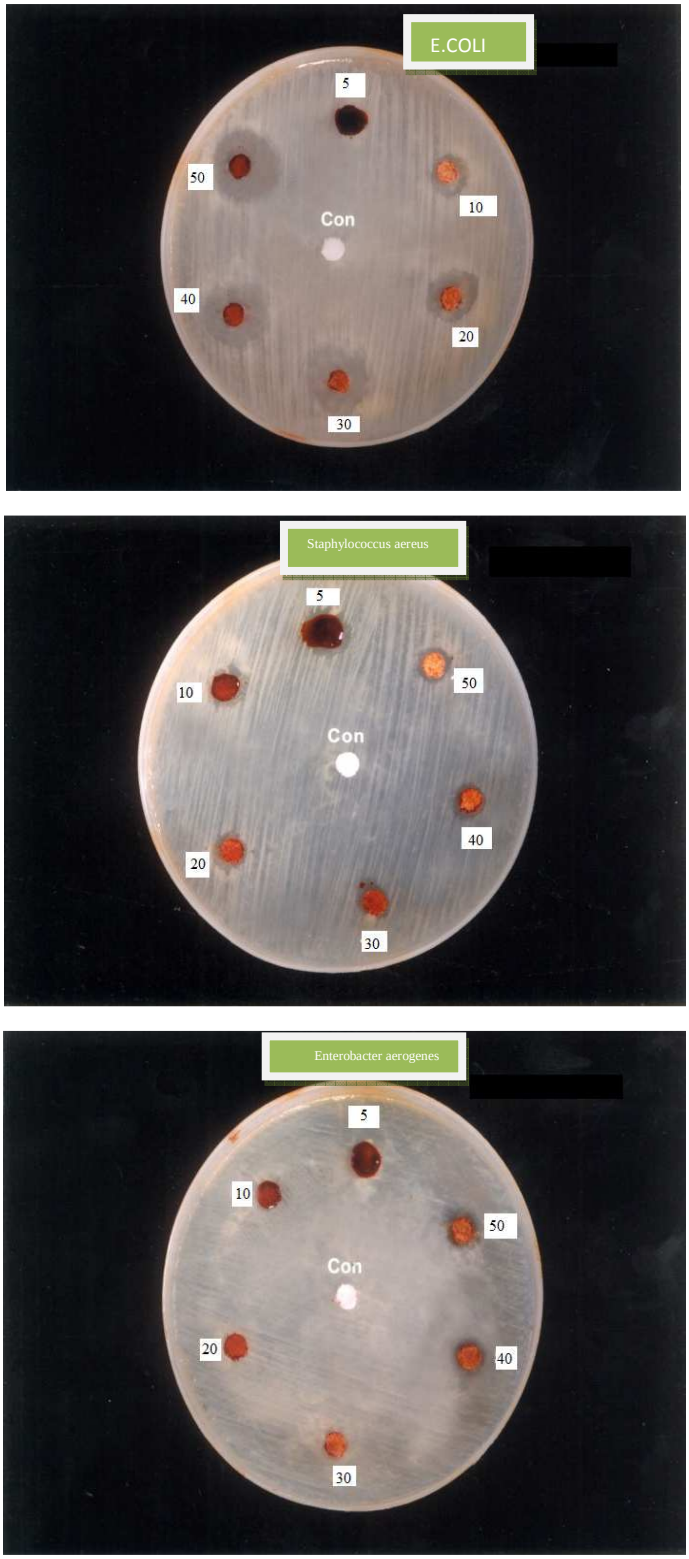



**Fig 5: Antibacterial activity of azo compound-I**

**Table 2 Antibacterial activity of azo compound II**

| 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 |
| *E.coli* | - | - | 3 | 2.8 | 2.5 | - |
| *Staphylococcus aereus* | - | - | - | - | 1 | 2 |
| *Enterobacter aerogenes* | - | - | - | - | - | 1 |

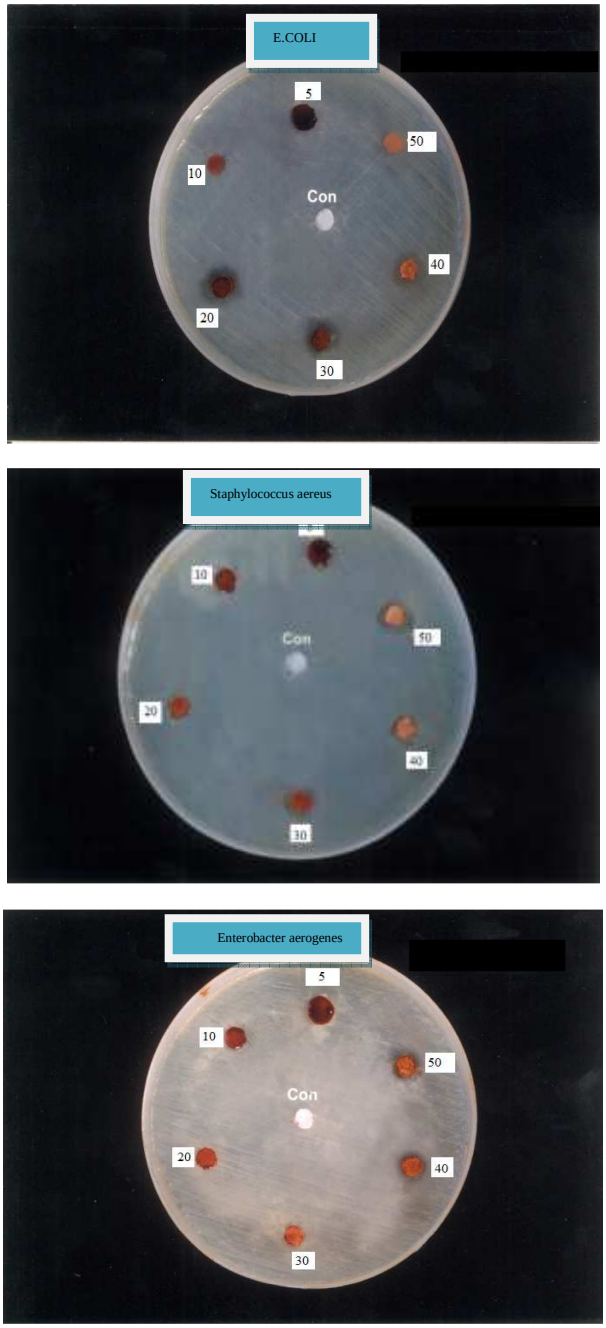



**Fig 6: Antibacterial activity of azo compound-II**

## CONCLUSION

The diazotized cardanol based dyes were successfully synthesized and characterized by using IR and $^{1}$H-NMR spectroscopy. On the basis of the results it can be concluded that azo compound I has significant antibacterial activity. It can be used for the development of new antibacterial drugs in the development of new pharmaceuticals.

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