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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4):490-497

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Antibacterial activity of azo compounds synthesized from the natural renewable source, cardanol

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ABSTRACT

Cashew nut shell liquid (CNSL), a byproduct of cashew nut processing industry contains mainly cardanol, a meta substituted phenol. Cardanol has been widely used as a synthon for the preparation of number of polymers and industrial products. p -sulphanilic acid and p-anisidine have been diazotised and coupled with cardanol to produce diazotised-p-sulphanilic acid cardanol dye and diazotised-p-anisidine cardanol dye respectively. They have been characterized by UV, FT- IR and ^{1}H-NMR spectroscopic techniques and their antibacterial activity has also been studied and reported for the first time.

Key words: cardanol, p-sulphanilic acid, p-anisidine, diazotisation, antibacterial activity.

INTRODUCTION

Synthesis of medicinally important compounds is very difficult and thus the cost of medicine is also high because of the non-availability of source materials especially aromatic compounds. The plants are the potential source of many drugs; but they did not yet get proper recognition. Many natural phenolic compounds found in plants and fruits are known to have antibiotic and antioxidant properties [1, 2]. Cashewnut shell liquid (CNSL), a byproduct of cashew nut industry, is a unique natural source of m-substituted unsaturated long chain phenol [3, 4]. Cashew nut shell liquid contains anacardic acid, cardol, 2- methyl cardol, and cardanol [5, 6]. Anacardic acid is known to exhibit antitumour [7], antimicrobial [8] and antiacne [9] properties. Cardanol finds many applications in the form of phenol – formaldehyde resin in varnishes, paints and brake linings [10]. Cardol is also active against the filarial parasite of cattle *Setaria digitata*

[11]. The cardanol and anacardic acid have antimicrobial activity against *Pseudomonas fluorescens* [12].

In the present study p-sulphanilic acid and p-anisidine are diazotized separately and then coupled with cardanol to produce diazotised-p-sulphanilic acid cardanol dye and diazotized-p- anisidine cardanol dye respectively. They are characterized by UV,

FT- IR, ^{1}H- 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, potassium hydroxide and methanol were received from M/s BDH Ltd (India). p-sulphanilic acid, p-anisidine were received from E. Merck (Germany). The chemicals were used as received.

Ultraviolet spectral analysis was carried out in a UV- VIS Double beam spectrophotometer 2201, Systronics. Infrared spectra were taken in a Shimadzu-FT-IR spectrophotometer by KBr pellet method. ^{1}H-NMR spectra were taken using Bruker Avance III 400 NMR spectrometer using $CDCl_3$ as the solvent.

Synthesis of azo compound I and azo compound II

p-sulphanilic acid (1.73g, 0.01mol) was dissolved in dilute hydrochloric acid, stirred vigorously while being cooled to 0^{o}C. A solution of sodium nitrite (0.70g, 0.01mol) in distilled water was added dropwise to the reaction mixture and the solution was kept below 0^{o}C. This solution was added dropwise to methonolic cardanol

(3g, 0.01mol) dissolved in KOH (0.56g, 0.01mol) with constant stirring to give reddish brown oil diazotised p-sulphanilic acid cardanol dye. The solution was cooled to 0^{o}C. The dye (azo compound I) was extracted from the reaction mixture by treating with methylene chloride. The methylene chloride layer was washed repeatedly 3-4 times with 20 ml of distilled water and evaporated to dryness. The yield was 80-85%. Similarly

p-anisidine (1.23g, 0.01 mol) was diazotised and coupled with cardanol to synthesise diazotised p-anisidine cardanol dye (azo compound II).

Determination of antibacterial assay

Bacterial strains used for testing included *Klebsiella pneumoniae* (NCIM 5082), *Pseudomonas aeruginosa* (NCIM 2026), *Solmonella typhi* (NCIM 2501), *Bacillus cereus* (NCIM 2703) and *Streptococcus pyogenes* (NCIM 2608).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, India) into 20 mm × 100 mm Petri plates. Inoculam (5 ml) 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 (1000 μg) of each azo compound was prepared by dissolving 10 mg of each compound separately in 10 ml of ethanol. 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 (200 μl) was placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. Standard antibiotic oflaxacin 5 μg /disc was used as standard for bacteria. 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). From the results, the activity index and proportion index were calculated using the following formulae.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

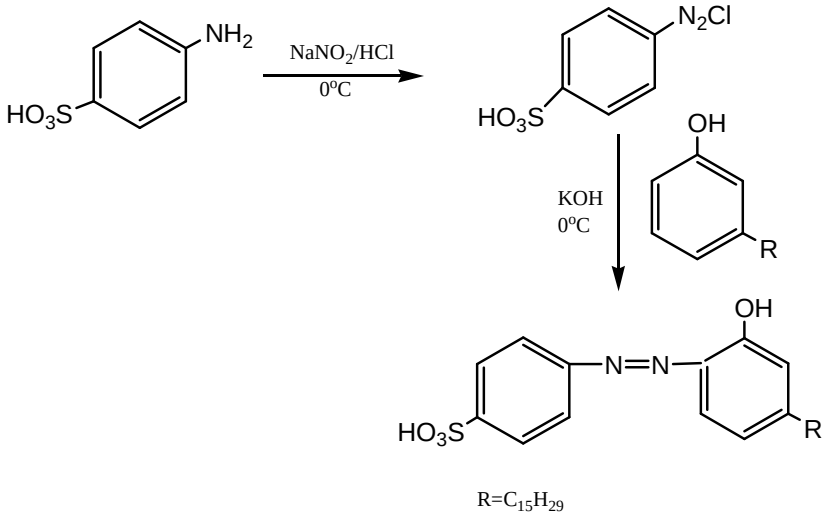
$$\text{Proportion index (PI)} = \frac{\text{Number of positive results obtained for individual compound}}{\text{Total number of tests carried out for each compound}}$$

RESULTS AND DISCUSSION

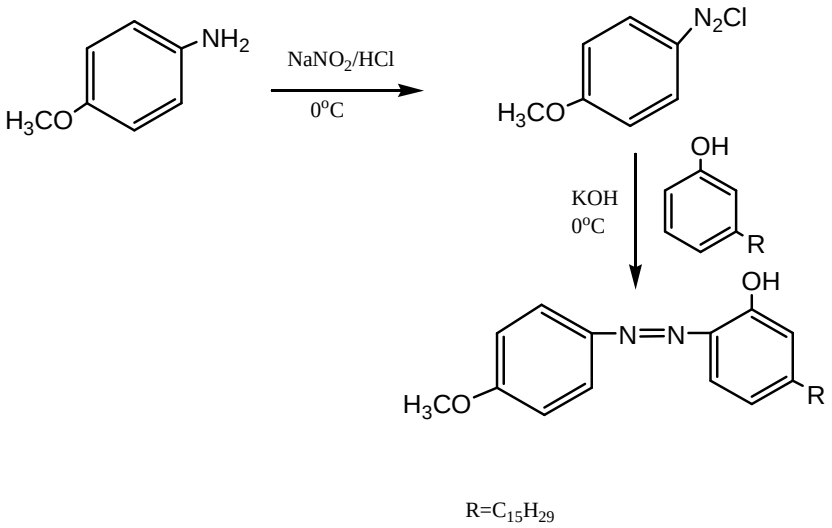
Mechanism for the synthesis of azo compound I and azo compound II

In the first step p-sulphanilic acid and p-anisidine 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 and 2.



Scheme 1. Synthesis of diazotised-p- sulphanilic acid cardanol dye



R=$C_{15}H_{29}$

Scheme 2. Synthesis of diazotized-p- anisidine cardanol dye

Characterization

UV-VIS and FT- IR Spectral analysis of azo compound I and azo compound II

In the UV –VIS spectroscopic analysis of azo compound I shows sharp peak at 230 nm which is due to π-π* transition of aromatic phenyl ring and the peak at 368 nm is due to n-π* transition of azo group. Similarly azo compound II shows peaks at 244 nm due to π-π* transition of aromatic phenyl ring and 358 nm due to n-π* transition of azo group.

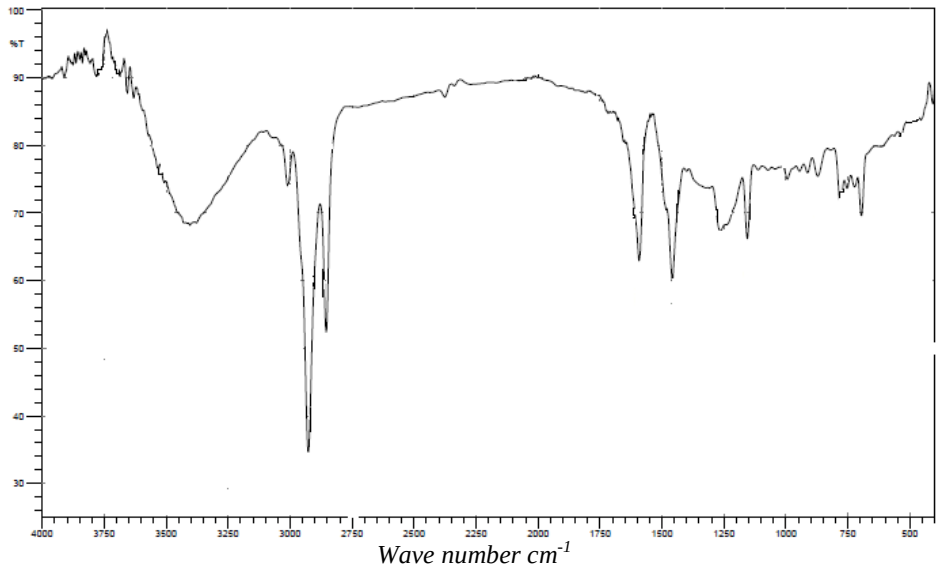
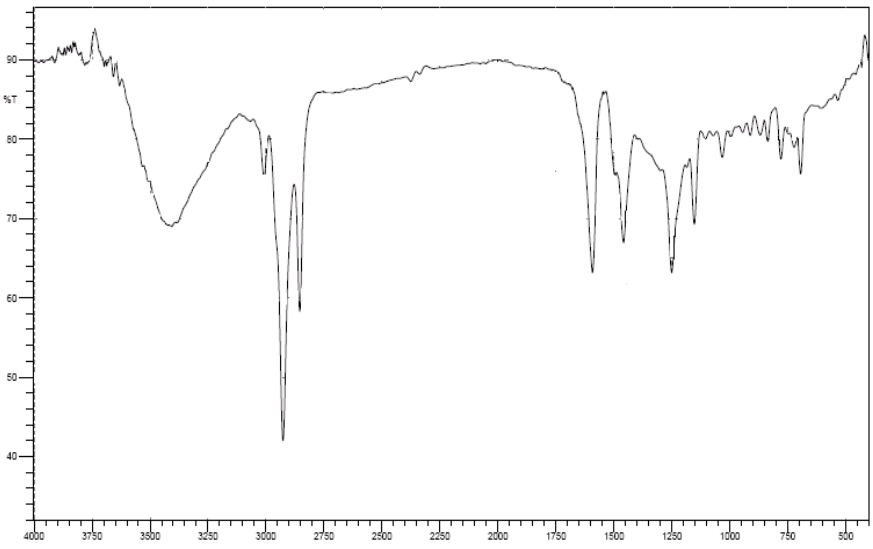


Fig. 1 FT-IR Spectrum of diazotized p- sulphanilic acid dye

In the Fig. 1, FT- IR spectrum of compound I, the 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. The peak at 1265 cm^{-1} shows the symmetric stretching of SO_3 group. In the Fig. 2, FT- IR spectrum of compound II, the peak

at 2924 cm^{-1} shows the symmetrical CH_2 stretching of the side chain of cardanol and the peak at 1458 cm^{-1} shows the presence of azo group.



Wave number cm^{-1}

Fig. 2 FT-IR Spectrum of diazotized p- anisidine cardanol dye

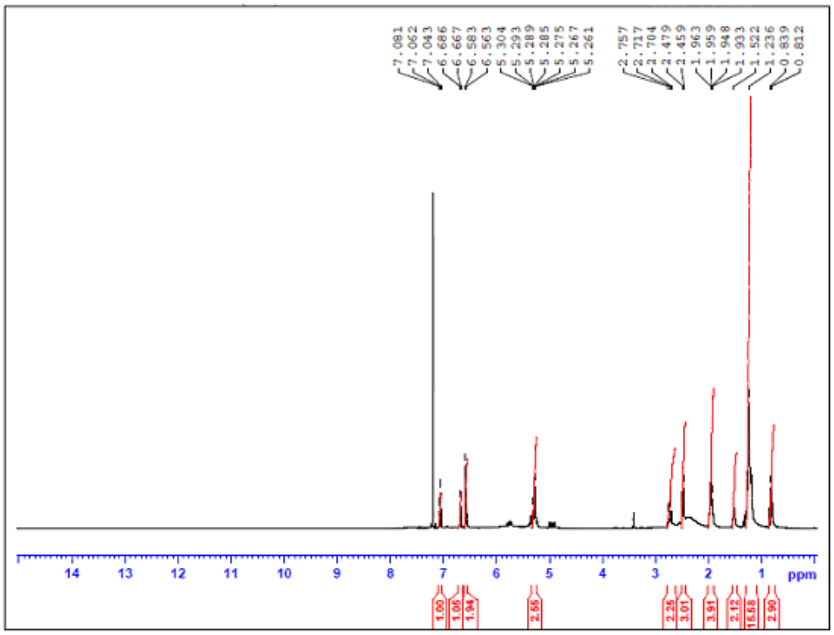


Fig. 3 ^{1}H- NMR spectrum of p-sulphanilic acid dye

^{1}H- NMR spectral analysis of azo compound I and azo compound II

^{1}H- NMR spectrum of azo compound I (Fig. 3) shows multiplet at 6.6-6.9 ppm is due to the aromatic protons of benzene nuclei of cardanol. The peak at 5.3 ppm is due to the phenolic hydroxyl proton. The multiplet at 7.0-7.2 ppm is due to the aromatic protons of sulphanilic acid. The peak at 1.3-2.5 ppm is due to the methylene group of the long alkyl side chain of cardanol.

The peak at 0.8 ppm is due to the terminal methyl group of the side chain of cardanol. In the ^{1}H-NMR spectrum of azo compound II (Fig.4) the peak at 6.4-6.8 ppm is due to the aromatic protons of benzene nuclei of cardanol. The peak at 5.2 ppm is due to the phenolic hydroxyl proton. The multiplet at 7.6-7.8 ppm is due to the aromatic protons of p-anisidine. The peak at 1.3-2.5 ppm is due to methylene protons of the long alkyl side chain of cardanol.

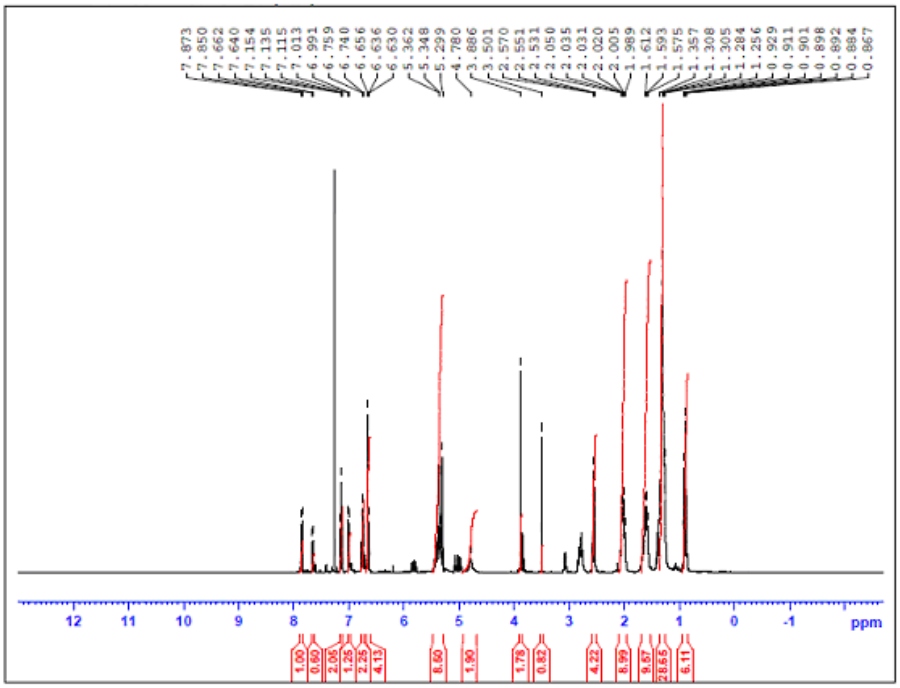


Fig. 4 ^{1}H-NMR spectrum of p-anisidine acid dye

Table 1 Antibacterial activity of azo compound I

Name of the organism	Zone of Inhibition (mm)										
	Concentration of azo compound I										Oflaxacin 5 µg
	80 µg		40 µg		20 µg		10 µg		5 µg		
	DZI	AI	DZI	AI	DZI	AI	DZI	AI	DZI	AI	
Klebsiella pneumoniae	22	0.85	20	0.77	20	0.77	18	0.69	17	0.65	26
Pseudomonas aeruginosa	-	-	8	-	7	-	7	-	-	-	-
Bascillus cereus	17	0.56	16	0.53	16	0.53	11	0.36	9	0.30	30
Solmonella typhi	-	-	-	-	-	-	8	0.47	7	0.41	17
Streptococcus pyogenes.	-	-	-	-	7	0.21	-	-	-	-	32

DZI – Diameter of zone of inhibition, AI – Activity Index

Antibacterial activity of azo compound I and 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 activitiy against the test organisms. The azo compound I shows the highest antibacterial activity with the inhibition zone of 22 mm against *Klebsiella pneumonia* at 80 µg concentration which is almost close to that of the standard ofloxacin (26

mm) and 17 mm against *Bascillus cereus* at the same concentration. The azo compound II shows only less antibacterial activity when compared with azo compound I. The results were also expressed by means of Activity Index (Table 1 and Table 2) and Proportion Index (Fig. 5).

Table 2 Antibacterial activity of azo compound II

Name of the organism	Inhibition zone diameter (mm)										
	Concentration of azo compound II										Oflaxacin 5 µg
	80 µg		40 µg		20 µg		10 µg		5 µg		
	DZI	AI	DZI	AI	DZI	AI	DZI	AI	DZI	AI	
Klebsiella pneumoniae	8	0.31	-	-	7	0.27	-	-	7	0.27	26
Pseudomonas aeruginosa	-	-	-	-	8	-	-	-	10	-	-
Bascillus cereus	9	0.30	7	0.23	7	0.23	7	0.23	7	0.23	30
Solmonella typhi	-		-		-		-		-	-	16
Streptococcus pyogenes.	-		-		-		-		-	-	34

DZI – Diameter of zone of inhibition, AI – Activity Index

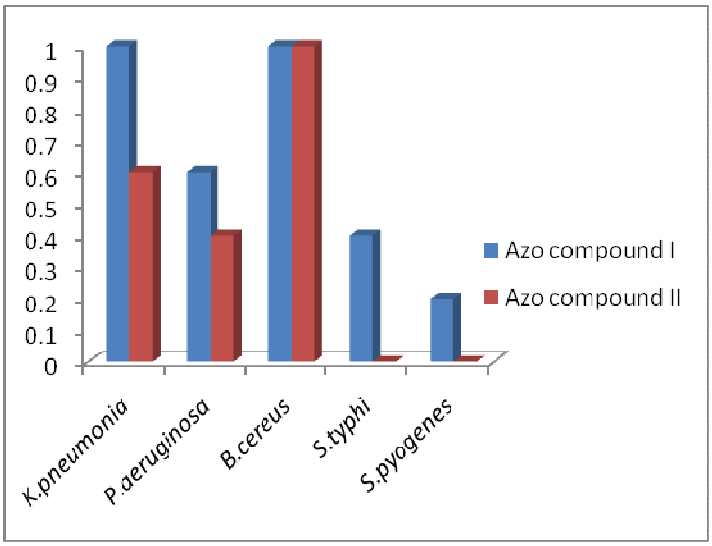


Fig. 5 Proportion Index of antibacterial activity of the azo compound I and azo compound II.

CONCLUSION

On the basis of the results it can be concluded that azo compound I has significant antibacterial activity. It can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals.

Acknowledgement
One of the authors N.T.Nevaditha thanks the University Grants Commission, New Delhi, and the Principal, Nesamony Memorial Christian College, Marthandam, Tamil Nadu for selecting her under FDP programme.

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