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Self-assembled cardanol azo derivatives as antifungal agent with chitin-binding ability

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ABSTRACT

Cardanol is a non-isoprenoic phenolic lipid-mixture of distilled cashew nut shell liquid obtained from *Anacardium occidentale*. Herein, cardanol is purified from cashew nut shell liquid (CNSL) and synthesized to new compounds with different azo amphiphiles. These synthesized compounds are allowed to self-assembled in hydrophobic environment and checked antifungal activity against *Candida albicans*. Self-assembled structure of CABA showed higher antifungal activity (16 μg/mL) and chitin-binding ability in comparison to CAP and CANB. Furthermore, the self-assembled azo amphiphiles are immobilized with silver ions to prepare hydrogel which showed eight folds enhanced antifungal activity. Toxicity is reduced by several folds of self-assembled or hydrogel structure in comparison to pure compounds. Thus, the self-assembled structure of amphiphiles and their hydrogels have been found to be new macromolecules of interest with potential use as antifungal drugs.

1. Introduction

Design and development of non-covalent self-assembly supramolecules from renewable resources have received remarkable attention due to their potential application as bioactive agents in tissue engineering to drug development [1,2]. Several studies have shown the potentiality of self-assembled value added materials as ranging from coating on medical device to antibacterial activity [3,2]. The increased incidences of multi-drug resistant fungal pathogens are of great threat in infection control. Numerous attempts have been made to design or identify novel antifungal compounds with unique characteristics. Fungal infections are often serious with an associated fatality rates ranging from 50% to 100%. Most fungal infections are caused by *Candida albicans*, of oral-gastro-intestinal track of man and other warm-blooded animals [4,5]. Chitin is a homopolymer of β1,4-*N*-acetylglucosamine (GlcNAc) and takes part in cell wall synthesis of almost all fungi [6–8].

Cardanol is one of the promising renewable natural resources, a waste from the cashew industry, obtained CNSL [9]. It is yellow to brown colored phenolic lipid carrying a C15 side chain at meta-position with various degrees of unsaturation, known as m-pentadecenylphenol (Fig. 1a). Distillate CNSL is a mixture of non-isoprenoid phenolic lipid, obtained from roasting shells which contains cardanol (60–65%) with cardol (15–20%) and other polymeric material (10%) [10]. The major structural advantages of cardanol having reactive phenolic group and unsaturated hydrophobic alkyl side chain at the meta-position of phenolic group. Due to this unique nature, cardanol and its derivative could be recognized as amphiphilic building block with supramolecular architecture [11].

Cardanol display several biological activities including antimicrobial [12,13] antioxidant [14,15] and antitumor [16] however strong cytotoxicity of this kind of compound limits its application as lack of biocompatibility [17,18]. In order to decrease the toxicity of cardanol derivatives and try to improve antifungal activity, our aim was extended to develop self-assembled supramolecular structure. In the present study, we have designed and synthesized cardanol-based three different functional polar azo amphiphiles, 4-[(4′-cardanyl)azo] benzoic acid (CABA), 4-[(4′-cardanyl)azo] phenol (CAP), 4-[(4′-cardanyl)azo] nitro benzene (CANB). The antifungal activities of all synthesized self-assembled compounds along with their silver immobilized hydrogel have

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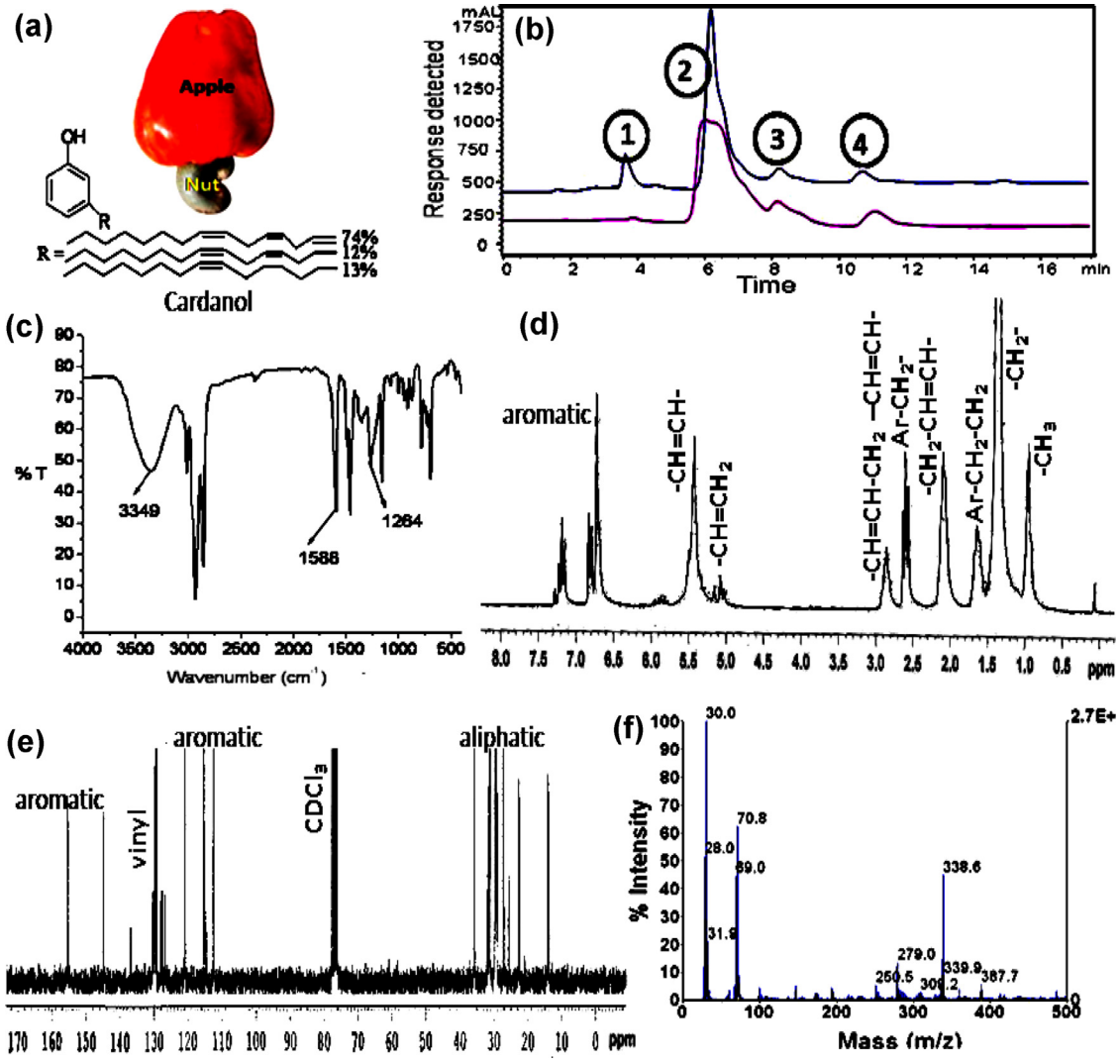

Fig. 1. Images for cardanol purification and characterization. Image of cashew fruit and chemical structure of cardanol (a), HPLC chromatogram of technical CNSL (blue) and pure cardanol (pink) (b). The peaks are mentioned in the chromatogram as cardol (1); triene (2); diene (3); and monoene (4). Analytical characterization of cardanol with FT-IR (c), ^{1}H NMR (d), ^{13}C NMR (e), MALDI-MS spectrum: [cardanol + potassium]$^{+}$ (f). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

been investigated against *C. albicans*, with chitin (*N*-acetyl-D-glucosamine) binding abilities.

2. Materials and methods

2.1. Chemicals and reagents

Technical standard Cashew nut shell liquid was purchased from Satya Cashew Industry (India). Para amino Benzoic acid, para amino phenol, para amino nitro benzene, sodium nitrite and sodium hydroxide were purchased from Sigma Aldrich (USA). Chitin, Triton X-100, potassium phosphate buffer, glutaraldehyde, Epon-Araldite resin and hydrogen peroxide were from Merk (India). All solvents were of analytical grade from Merck (India).

2.2. Instrumentation

2.2.1. High performance liquid chromatography (HPLC)

The HPLC analysis was done on an Agilent HPLC system (1100 series), comprising of two reciprocating pumps, a 481 variable-wavelength detector, an injector with 20 μL loop, and ZORBAX SB C-18 (4.6 mm × 150 mm, particle size 5 μm) Agilent column was used. The mobile phase was acetonitrile/water/acetic acid (80:20:1) at a flow rate of 1 mL/min. Absorbance was monitored at 250 nm. Each analysis was carried out of 5 mg/mL in acetonitrile and filtering through a C18 Sep-Pak cartridge (Water Associates, Milford, MA).

2.2.2. Ultraviolet–visible spectroscopy (UV–vis)

The UV–vis absorbance spectra were scanned (300–600 nm) and recorded on a Shimadzu UV-1601 against only solvent as blank reference. Experiments are performed by keeping concentration 0.02 mM samples. In all experiments, solutions were taken in quartz cuvette of 1-cm path length.

2.2.3. Fourier transform infrared (FTIR)

For IR experiment, samples were dissolved in chloroform and placed onto a KBr pellet and dried. The dried specimen was recorded on Shimadzu 8400 FT-IR spectrophotometer. Absorbance spectra were obtained from 4000 to 400 cm^{-1} with a 4 cm^{-1} resolution, Background spectra were also collected and subtracted.