

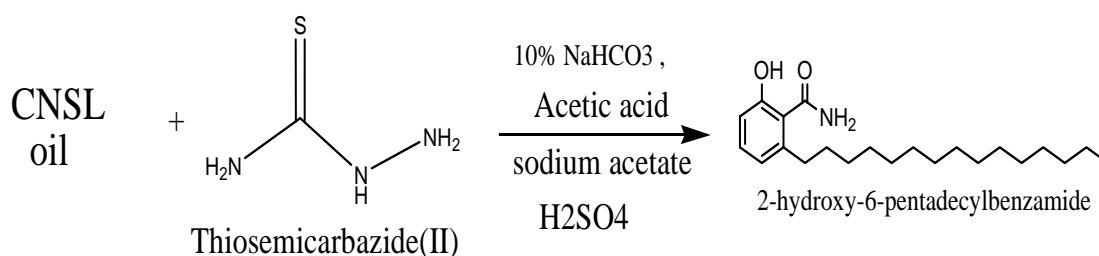
Antibacterial Activity of 2-Hydroxy-6-Pentadecylbenzamide Synthesized from CNSL Oil

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Summary

The aim of study is synthesis of 2-hydroxy-6-pentadecylbenzamide from Cashew-nut Shell Oil with reference to antibacterial activity. CNSL oil mixed with NaHCO₃ for half an hour, gives two layers (NaHCO₃ layer and oil layer) they are separated by centrifuge. NaHCO₃ layer is acidified with 1:1 HCl. NaHCO₃ layer is refluxed with Thiosemicarbazide (II) in presence of Acetic acid and H₂SO₄ to form 2-hydroxy-6-pentadecylbenzamide. The structure is confirmed by NMR and Mass spectroscopy. The homogeneity and purity of the compound are checked by T.L.C. and their antibacterial activity is evaluated by Agar well diffusion method.(19,20)



Keywords: Cashew - nut Shell Oil, Thiosemicarbazide (II), 2-hydroxy - 6 - pentadecylbenzamide, H₂SO₄

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Introduction

Cashew nut is regarded as lost crop in the content of agricultural produce of India instead of its industrial and export potentials. The major by-product of cashew nut is the liquid obtained from the pericarp known as cashew nut shell liquid (CNSL). (24-25) CNSL is one of the sources of naturally occurring phenols. (21-23) CNSL is amber-colored, poisonous, viscous oil obtained from the by-product shells of the cashew nut by extraction. It is often considered as the better and cheaper source of unsaturated phenols. CNSL has innumerable applications in polymer based industries, such as friction linings, paints, vanishes, laminating resins, rubber compounding resins, polyurethane based polymers, surfactants, epoxy resins, wood preservatives. It offers much scope and varied opportunities for the development of other tailored polymers. More so, resins based on the reaction products of cardanol, phenol and formaldehyde is used to improve the resistance of rubber articles to cracking and ozone degradation.

There are three different methods generally used in extracting cashew nut shell liquid from cashew nuts, namely mechanical, roasting and solvent extraction. The processes used are mainly hot-oil and roasting in which the CNSL oozes out from the shell. The traditional method of extracting CNSL is by roasting the nuts over an open fire. This removes the CNSL by charring or degradation there by wasting the liquid which is a valuable source of natural phenols (26-27). CNSL, if properly extracted, has a lot of industrial applications.

In present study 2-hydroxy-6-pentadecylbenzamide obtained from Cashew-nut Shell Oil and checked for its antibacterial activity.

Materials and Methods

Chemicals: Thiosemicarbazide (II), 2-hydroxy-6-pentadecylbenzamide, H₂SO₄

Method: The homogeneity and purity of the compound were checked through T.L.C, ¹HNMR and Mass spectroscopy by DMSO on JEOL-FX-100 Spectroscopic methods.

Synthesis of 2-hydroxy-6-pentadecylbenzamide

Cashew-nut Shell Oil is obtained by extraction method from cashew nut shell. It is mixed with NaHCO₃ for half an hour; it gives two layers. These layers (NaHCO₃ layer and oil layer) are separated by centrifugation. NaHCO₃ layer is acidified with 1:1 HCl to obtain aqua's solution. 5.4 ml aqua's solution is dissolved in 20 ml Acetic acid. 1.4gm of Thiosemicarbazide (II) is dissolved completely in 20 ml Acetic acid in presence of 0.5 g sodium acetate. In Thiosemicarbazide (II) solution 20 ml 18% H₂SO₄ aqua's solution is added slowly with constant stirring and Thiosemicarbazide (II) mixture is added drop by drop in aqua's solution. This mixture is refluxed for one hour at 90 °C temperature with constant stirring. During progress of this reaction the fumes of acetic acid get removed after 2 hours, then stop the heating and cool this reaction mixture at room temperature, the reaction mixture is allowed to stand for eight hours for settling. Gray colored crystals are separated which are then washed with distilled water and dried. The product obtained is characterized by Spectroscopic methods. The product obtained is found to be 2-hydroxy-6-pentadecylbenzamide with M.P. 345 °C, the yield is 83 %.

Disc Diffusion Bioassay

Antibacterial Activity of the 2-hydroxy-6-pentadecylbenzamide obtained by synthesis of Cashew-nut Shell Oil, Thiosemicarbazide (II) was studied against bacterial strains by the agar well diffusion method. Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. 2-hydroxy-6-pentadecylbenzamide obtained from synthesis of Cashew-nut Shell Oil, Thiosemicarbazide (II) was diluted in 100% dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL and 2.5 mg/mL. The antibacterial activity was evaluated at two different concentrations viz. 500 µg/ well and 250 µg/ well. The Mueller Hinton agar was melted and cooled to 48 - 50°C and a standardized inoculum (1.5×10^8 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (8.5 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The experiment was performed three times to minimize the error and the mean values are presented.

Results

¹HNMR

0.83(t,3H),1.26(m 26 H one beta & one alpha),2.55(2H ,t),9.83(1H aromatic OH),7.85(NH₂, p-amine),6.86(1H,d), 7.29(1H,dd), 6.73(1H,d).

MASS : 347.28, 348.29, 349.29.

Table 1. Antibacterial activity of 2-hydroxy-6-pentadecylbenzamide from Cashew-nut Shell Oil by agar well diffusion method.

Microorganisms	<u>Inhibition Zone (mm)*</u> 2-hydroxy-6-pentadecylbenzamide
<i>Staphylococcus aureus</i> (NCIM No. 5021, ATCC No. 25923)	35 mm
<i>Escherichia coli</i> (NCIM No: 2931, ATCC No. 25922)	29 mm

*: values include cup borer diameter (8.5 mm) and are mean of three replicates.

Discussion

2-hydroxy-6-pentadecylbenzamide is easily synthesized from aqua's solution which is obtained from CSNL. Thiosemicarbazide (II) reacts in presence of Acetic acid, H₂SO₄ and Sodium acetate, distilled water is used as a medium. The structure is confirmed by ¹H NMR and MASS spectroscopic data. The antibacterial activity was found to be good evaluated by Agar well diffusion method. 2-hydroxy-6-pentadecylbenzamide has highest antibacterial activity. The results of the antibacterial study (Table 2) shows that this tested compound inhibits the growth of *Staphylococcus aureus* (NCIM No. 5021, ATCC No. 25923), and *Escherichia coli* (NCIM No: 2931, ATCC No. 25922) bacteria. It inhibits the growth of both Gram positive and Gram negative bacteria at lower concentration.

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