STRUCTURE ELUCIDATION OF A PURE ORGANICCOMPOUND ISOLATED FROM THE BARK OF *ADINA CORDIFOLIA* HOOK.F (HNAW)

Kyu Kyu Maw,[1] Hla Myo Min,[2] Myint Myint Sein[3]

Abstract

One of Myanmar indigenous medicinal plant, *Adina cordiforlia* Hook.f (Hnaw) was selected for chemical analysis. A pure organic compound was isolated from the bark of Hnaw by using advanced separation methods such as Thin Layer and Column Chromatography. The yield percent of this pure green crystal compound was found to be 2.35 % based upon the ethyl acetate crude extract. The pure isolated organic compound responds medium activity on *Bacillus pumilus* and *E. coli* and low activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The molecular formula of the pure compound was determined as $C_{22}H_{22}O_8$ by using some spectroscopic techniques such as FT IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DQF-COSY, HMQC, HMBC and EI-Mass spectral data. Hydrogen deficiency index of this compound is 12. Finally, the complete structure of this pure compound as dimer of 2-hydroxy-4-ethoxy cis-coumaric acid was elucidated by employing DQF-COSY, ^{1}H NMR splitting patterns, coupling constant (J- value) and HMBC spectroscopic studies.

Keywords: *Adina cordiforlia* Hook.f, Thin Layer and Column Chromatography, Spectroscopic techniques

Introduction

In ancient times, medicinal plants have been used all over the world as unique sources of medicines. Many medicinal plants represent a rich source of drugs individually or in combination have been recommended in various medical treaties for the cure of different diseases (Kirtikar and Basu, 1933).

Thousands of indigenous plants have been used by man from prehistoric times on all continents for relieving and curing ailments. In spite of tremendous development in the field of allopathy medicinal plants and their derivatives still remain one of the major sources of drugs in modern and traditional systems throughout the world playing a major role in medicinal therapy. In India about 7300 plant species are used in traditional health care systems. 90% of the medicinal plants which find place in day to day uses, many of these, are used as herbal remedies. The expanding domestic and global demand of herbal remedies. The expanding domestic and global demand of herbal products has put the native medicinal plant resources under significant stress (Kumari, 2013).

In this study, a pure bioactive organic compound was isolated from the bark of *Adina cordifolia* Hook.f belonging to the family, Rubiaceae, locally known as Hnaw by applying modern separation methods such as Thin Layer and Column Chromatography. Furthermore, the structure of this bioactive compound was elucidated by using some modern spectroscopic methods such as FT IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DQF-COSY, HMQC, HMBC and EI-Mass spectrometry.

[1] Dr, Lecturer, Department of Chemistry, University of Magway
[2] Dr, Professor, Department of Chemistry, University of Mandalay
[3] Dr, Professor (Retd.), Department of Chemistry, University of Mandalay

Botanical description of *A. cordifolia* (Figure 1) was shown as follows:


Figure 1 Plant and bark of *Adina cordifolia* Hook.f (Hnaw)

Family	-	Rubiaceae
Botanical name	-	*Adina cordifolia* Hook.f.
Myanmar name	-	Hnaw
English name	-	Yellow Teak
Medicinal uses	-	inflammation, urinary retention, wounds and ulcers, skin diseases, infection, dysentery, fever and burning sensation

Materials and Methods

The following advanced instruments were used in the characterization of the sample as well as in the structure elucidation of the pure compound.

1. UV lamp (Lambda 40, Perkin Elmer Co, England)
2. FT IR spectrometer (Shimadzu, Japan)
3. NMR spectrometer (500 MHz) (JEOL, Japan)
4. EI-Mass spectrometer

The chemicals used were analytical grade reagents. They were produced from British Drugs House (BDH) London and Merck, Germany. Analytical preparative thin layer chromatography was performed by using precoated silica gel (Merck, Co. Inc. Kieselgel 60 F_{254}). Silica gel (70 to 230 mesh ASTM) was used for column chromatography.

Sample Collection

The bark of Hnaw for experiment was collected from Bu-ta-lin Township, Sagaing Region, Myanmar. The samples were cut into small pieces and allowed to air dry. Then the dried pieces were stored in a well-stoppered bottle and used throughout the experiment.

Extraction and Isolation of Pure Organic Compound

Air dried sample (800 g) was percolated with 95 % ethanol (2.7 L) for two months. The extracted solution was filtered and evaporated in air. Then, it was re-extracted with ethylacetate (200 mL) and resulting solution was evaporated. The ethylacetate crude extract (6.5 g) was obtained.

The ethylacetate crude extract (6.5 g) was fractionated by column chromatography over silica gel with various ratios of n-hexane and ethylacetate from non-polar to polar. Totally (283) fractions were obtained. Each fraction was checked by TLC. The same R_f value fractions were combined. Three combined fractions were collected. Major combined fraction (II) gave only one spot on TLC and UV active. Pure green crystal (0.1526 g) was obtained. The yield percent of this pure compound (KKM-1) was found to be 2.35 % based upon the ethylacetate crude extract.

Screening of Antimicrobial Activities of the Pure Isolated Compound

Antimicrobial activities of the pure isolated compound were determined by agar-well diffusion method on six tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli* respectively.

Identification of the Pure Isolated Compound

The pure isolated compound was identified by modern spectroscopic techniques such as FT IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DQF-COSY, HMQC, HMBC and EI-Mass spectrometry.

Results and Discussion

Screening of Antimicrobial Activities of the Pure Isolated Compound

Antimicrobial activities of the pure isolated compound were determined by agar-well diffusion method on six tested organisms. The pure isolated organic compound responds medium activity on *Bacillus pumilus* (16 mm) and *E.coli* (18 mm) and low activities against *Staphylococcus aureus* (13 mm), *Pseudomonas aeruginosa* (12 mm) and *Candida albicans* (12 mm). The results are shown in Table 1 and Figure 2.

Table 1 Antimicrobial Activities of the Pure Isolated Compound

Sample	Solvent	Inhibition zone diameters (mm) against different microorganisms					
		I	**II**	**III**	**IV**	**V**	**VI**
Pure Compound	EtOH	-	13 (+)	12 (+)	16 (++)	12 (++)	18 (++)

Agar well~10 mm
10 mm~14 mm(+)
15 mm~19 mm(++)
20 mm above (+++)

Organisms
I. *Bacillus subtilis*
II. *Staphylococcus aureus*
III. *Pseudomonas aeruginosa*
IV. *Bacillus pumilus*
V. *Candida albicans*
VI. *E. coli*

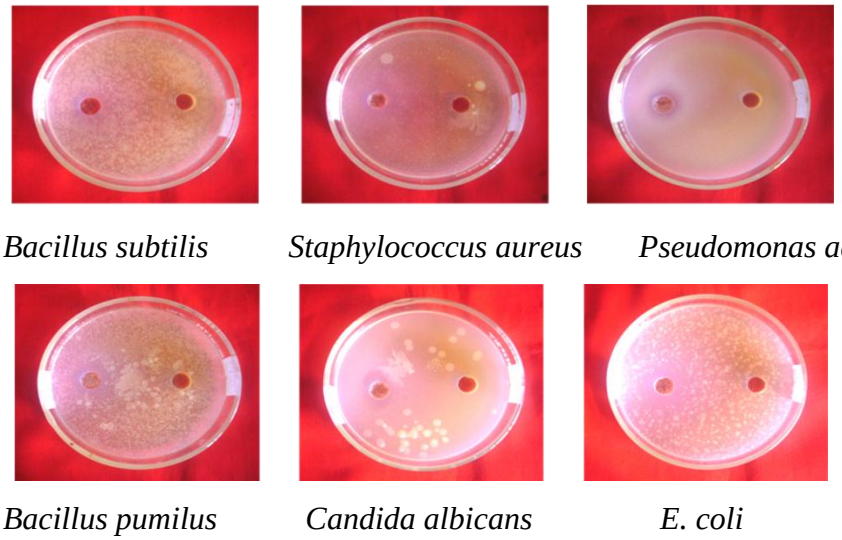


Figure 2 Screening of antimicrobial activities of the pure isolated compound

Identification of the Pure Isolated Compound

The ethyl acetate crude extract was separated by chromatography on a silica gel column using n-hexane and ethyl acetate with various ratios from non-polar to polar to obtain pure organic compound, dimer of 2-hydroxy-4-ethoxy cis-coumaric acid.

Molecular Formula of the Pure Isolated Compound

The molecular formula of the pure isolated organic compound could be determined by some spectroscopic methods such as FT IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DQF-COSY, HMQC, HMBC and EI-Mass spectrometry (Figure 3 to Figure 8).

According to FT IR spectrum (Figure 3) the characteristic bands at 3170 cm^{-1}, 2962 cm^{-1}, 2913 cm^{-1}, 1681 cm^{-1}, 1604 cm^{-1}, 1566 cm^{-1}, 1404 cm^{-1}, 1234 cm^{-1}, 1130 cm^{-1}, 1033 cm^{-1}, 829 cm^{-1} and 756 cm^{-1} showed that the pure isolated compound contains –OH group, sp^3 hydrocarbon, carbonyl group, aromatic benzenering, allylic hydrocarbon, gemdimethyl group, C–CO – O stretching vibration, C–C–O stretching vibration of alcohol group, C–O–C stretching vibration of ether group, trans or E and cis or Z alkenic groups, respectively (Silverstein *et al*., 2005).

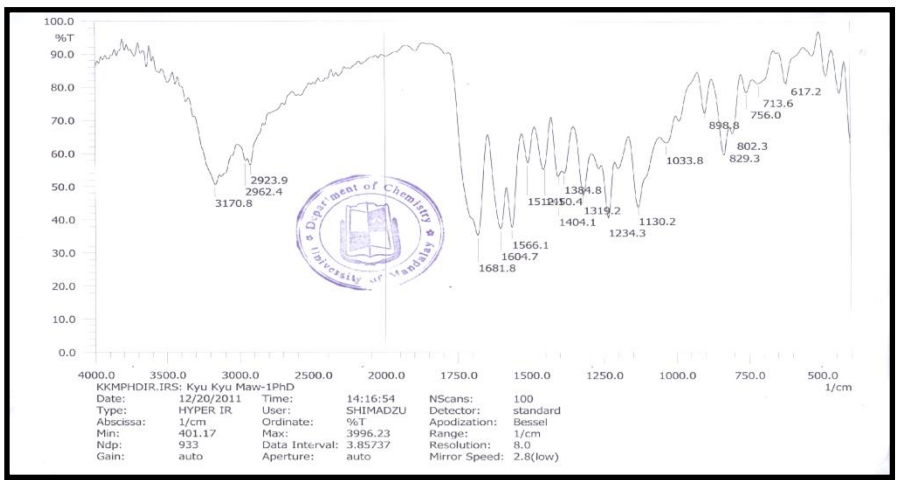


Figure 3 FT IR spectrum (KBr) of the pure isolated compound

^{1}H NMR (500 MHz) spectrum (Figure 4) represents the chemical shift values, splitting pattern and coupling constant (J-values) of protons. According to this spectrum, the pure isolated compound contains 9 protons (Timothy, 1999).

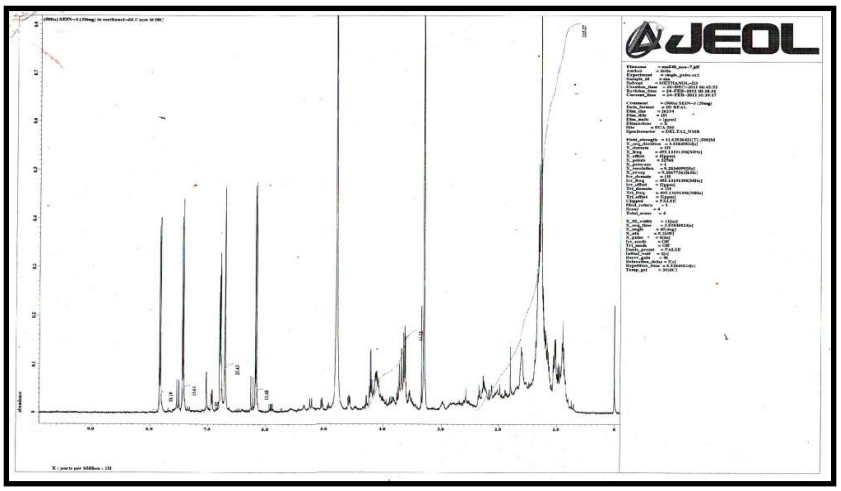


Figure 4 ^{1}H NMR spectrum (500 MHz, MeOH) of the pure isolated compound

^{13}C NMR spectrum (Figure 5) represents the total number of carbons. According to this spectrum, the pure isolated compound contains 11 carbons.

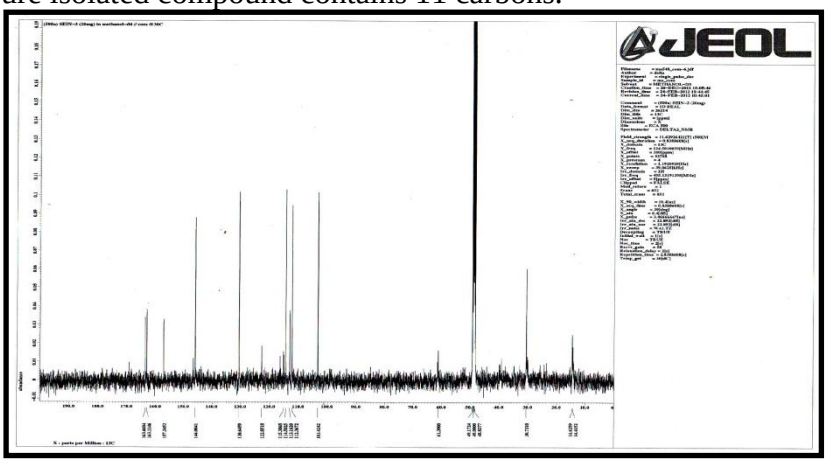


Figure 5 ^{13}C NMR spectrum(125 MHz, MeOH) of the pure isolated compound

The DEPT spectrum (Figure 6) confirms the number of carbons, protons and kinds of carbons containing in this compound (Silverstein *et al*., 2005).

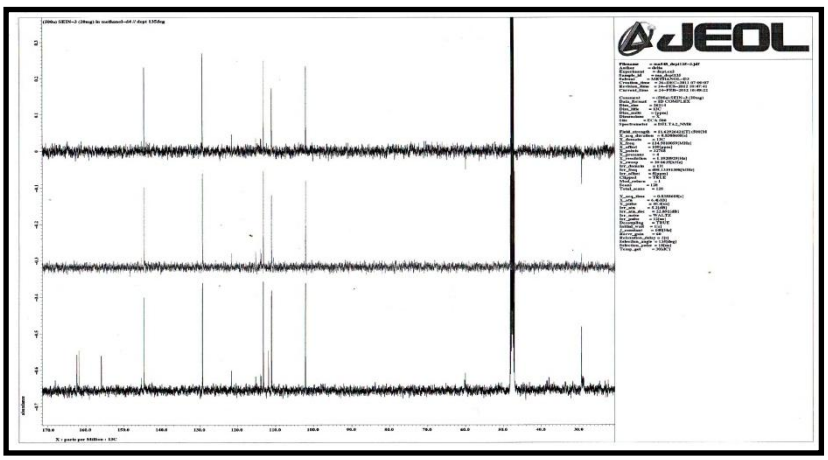


Figure 6 DEPT spectrum of the pure isolated organic compound

The HMQC spectrum (Figure 7) gives rise to the proton- carbon direct correlation (Timothy, 1999).

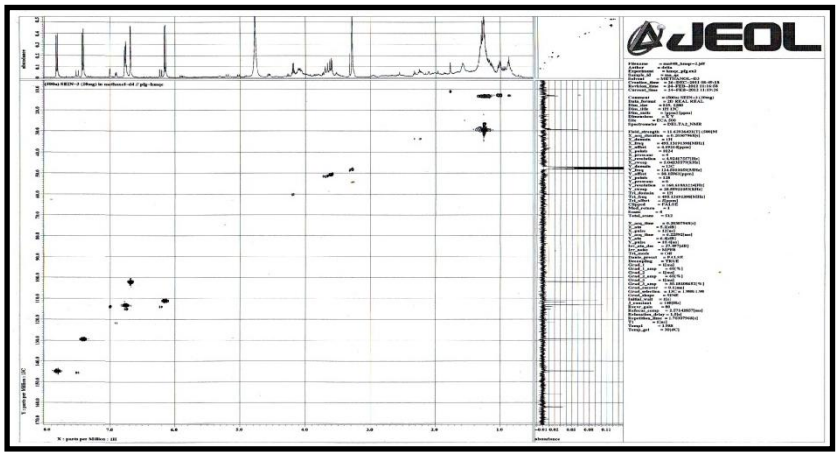


Figure 7 HMQC spectrum of the pure isolated organic compound

In addition EI-MS spectrum (Figure 8) of this compound shows the molecular ion peak at m/z 414 which implies the molecular mass of compound (Porter and Baldas, 1971).

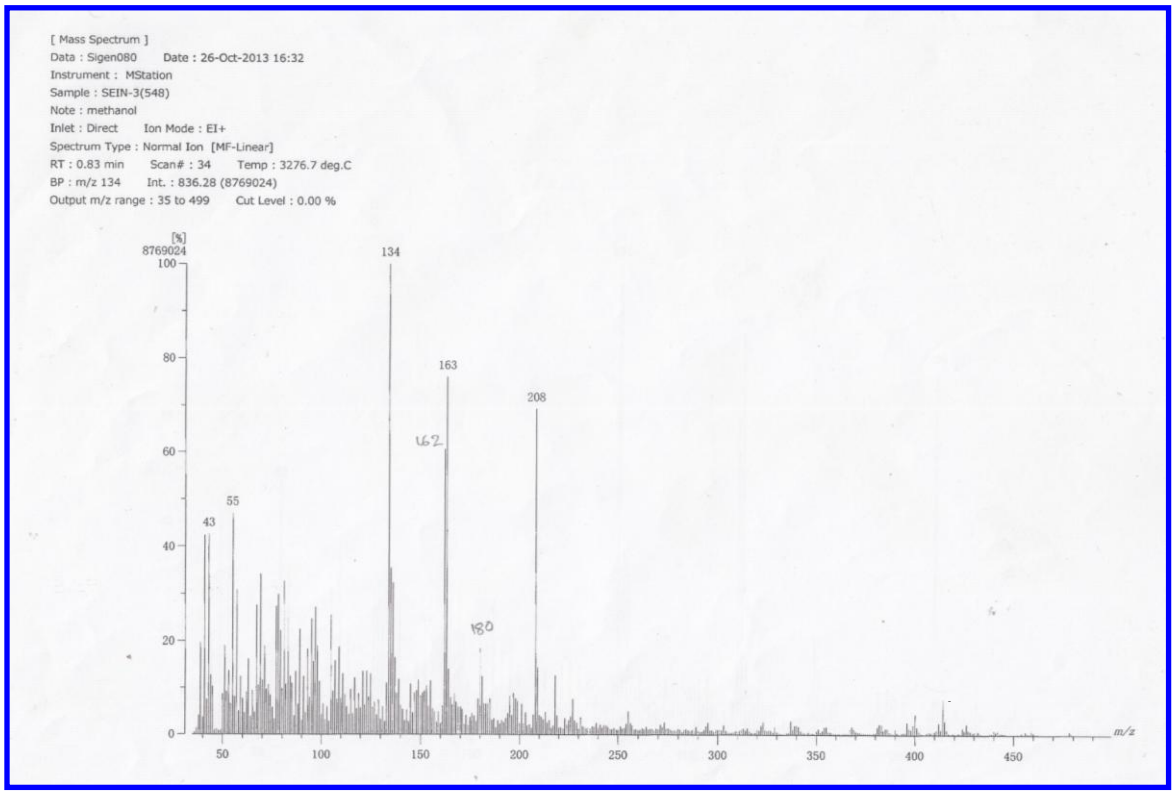


Figure 8 EI-MS spectrum of the pure isolated organic compound

According to ^{1}H NMR and ^{13}C NMR spectral data, the partial molecular formula is $C_{11}H_9$ and the partial molecular mass is 141.

In accordance with FT IR assignment, the pure compound should consist of at least one carbonyl group, one -OH group and one ether functional group.

Hence, the extended molecular formula is $C_{11}H_{10}O_3$ and extended partial molecular mass is 190.

However, in EI-Mass spectrum, the molecular ion peak m/z at 414 which represents the molecular mass of this pure compound. According to EI-Mass spectrum and the previous spectrums (^{1}H NMR , ^{13}C NMR, DEPT and HMQC), the compound is suggested to exist as a dimer.

Therefore, the extended molecular formula become to be $C_{22}H_{20}O_6$ and its molecular mass is 380.

Hence, the remaining partial molecular mass = 414 – 380 = 34. It should be two –OH groups.

Thus, the real molecular formula of this pure compound (KKM- 1) is $C_{22}H_{22}O_8$ which agrees with the nitrogen rule.

$$\text{Hydrogen Deficiency Index (HDI)} = 22 - \frac{22}{2} + 1$$

$$= 12$$

Structure Elucidation of the Pure Isolated Compound

The structure elucidation of the pure isolated compound could be determined by ^{1}HNMR, DQF-COSY, HMQC and HMBC spectral data (Figure 9 and Figure 10).

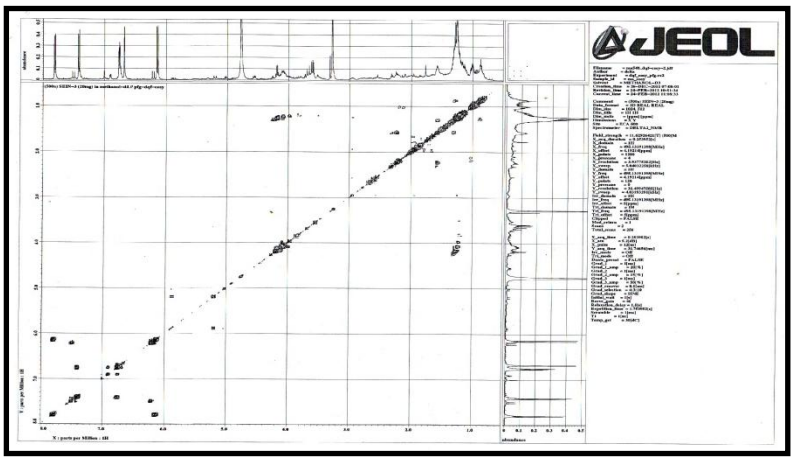


Figure 9 DQF-COSY spectrum of the pure isolated organic compound

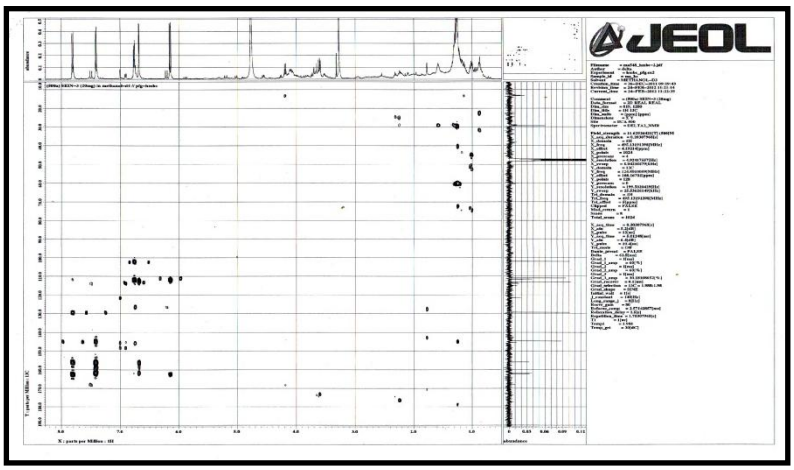
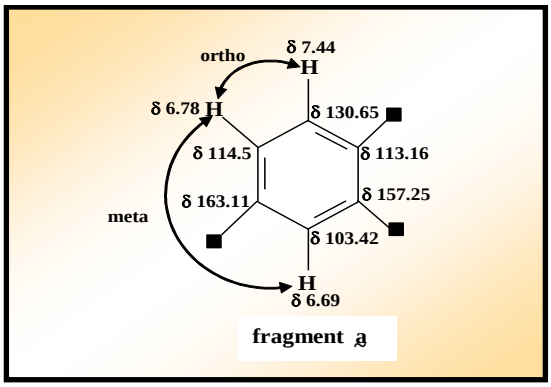
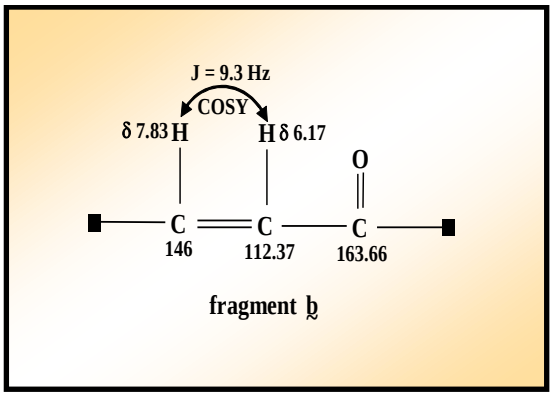


Figure 10 HMBC spectrum of the pure isolated organic compound

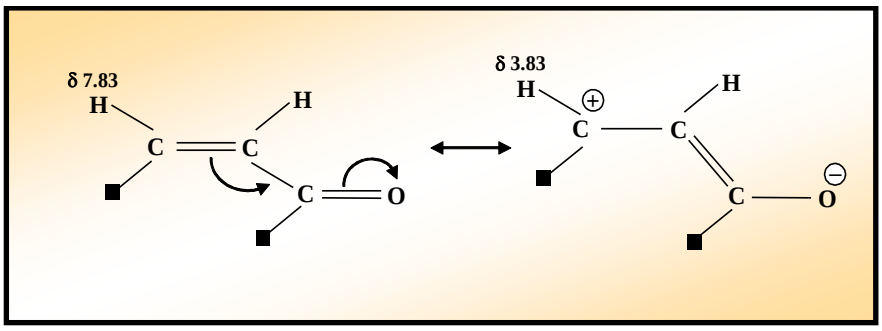
The substituted benzene fragment could be assigned by DQF-COSY (Figure 9), HMQC (Figure 7) and HMBC (Figure 10) spectra respectively. In the fragment a , the ortho orientation of two aromatic protons (δ 6.78 ppm and δ 7.44 ppm) and the meta orientation of two aromatic protons (δ 6.78 ppm and δ 6.69 ppm) could be confirmed by ^{1}H NMR splitting patterns and coupling constants J values.



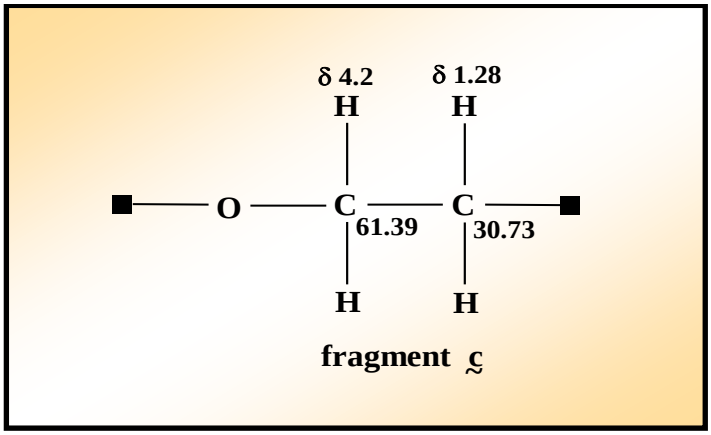
On the other hand, the alkenic fragment could be assigned by DQF-COSY, HMQC, HMBC spectra. It can be confirmed by ^{1}H NMR splitting pattern and coupling constant (J values) which indicates that the two alkenic protons (δ 7.83 ppm and δ 6.17 ppm) exist as cis position to each other (Fragment b).



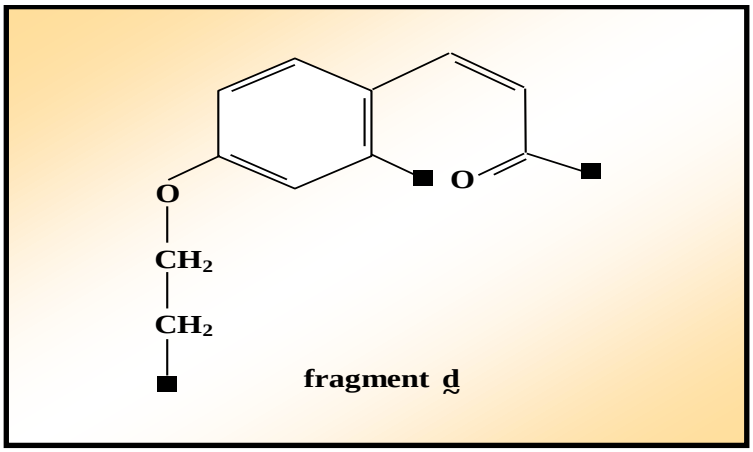
The observation of downfield chemical shift of one of alkenic protons (δ 7.83 ppm) is consistant with the theoretical resonance contribution structures caused by carbonyl group next to them, as shown below.



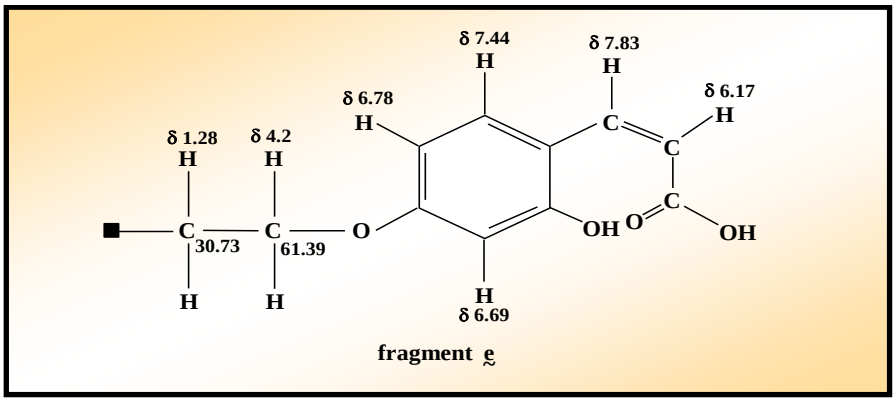
Moreover, the ethylenylhydroxy (–O–CH_2–CH_2–) fragment could be elucidated by DQF-COSY and HMQC spectra (Fragment c).



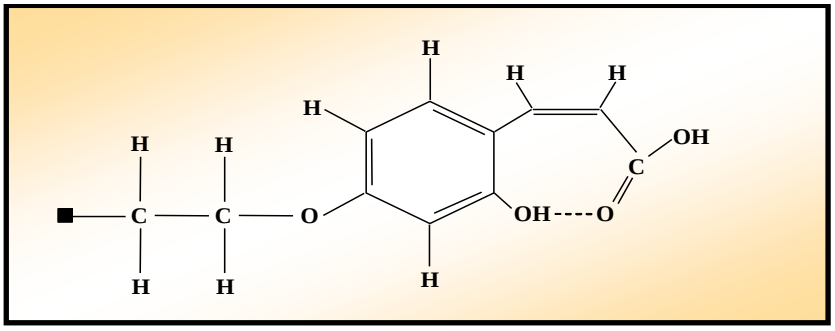
The combination of fragments a, b and c gives the gross molecular structure (fragment d̲) shown below.



According to the FT IR spectrum, the reasonable attachment of two hydroxy groups to the downfield chemical shift aromatic quaternary carbon (δ 157.25 ppm) and the carbonyl carbon (δ 163.66 ppm) produces the following fragment e.

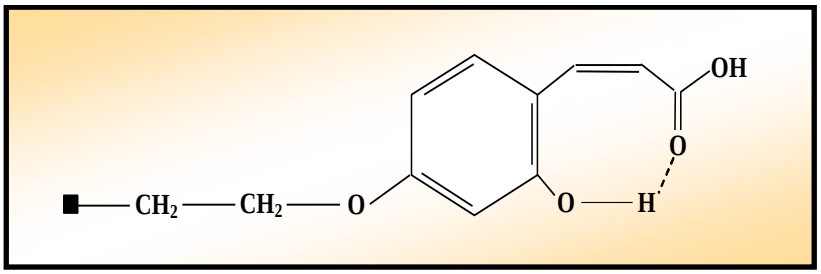


In the FT IR spectrum, the unusual lower frequency of –OH functional group at 3170.8 cm^{-1} and the carboxylic carbonyl group at 1681.8 cm^{-1} is assigned to be formation of intramolecular hydrogen bonding in this fragment, as described below.

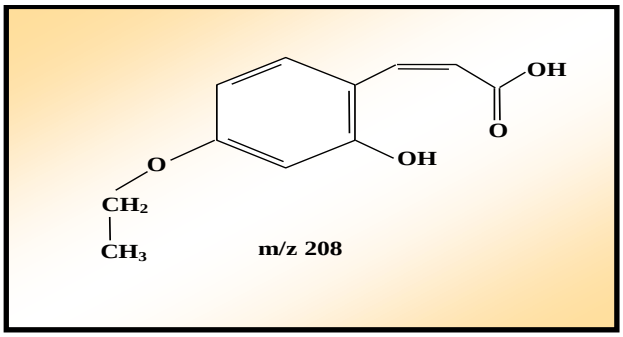


There are anomalous stretching frequencies of –OH and – CO – groups observed in FT IR spectrum. Carboxylic group – OH shows normal pattern (broad) and position (between 3200 – 2500 cm^{-1}). But phenolic – OH shows very much lower frequency 3170.8 cm^{-1}. At the same time, carboxylic carbonyl also shows very lower frequency value 1681.8 cm^{-1} if it is compared with its normal value 1740 cm^{-1}.

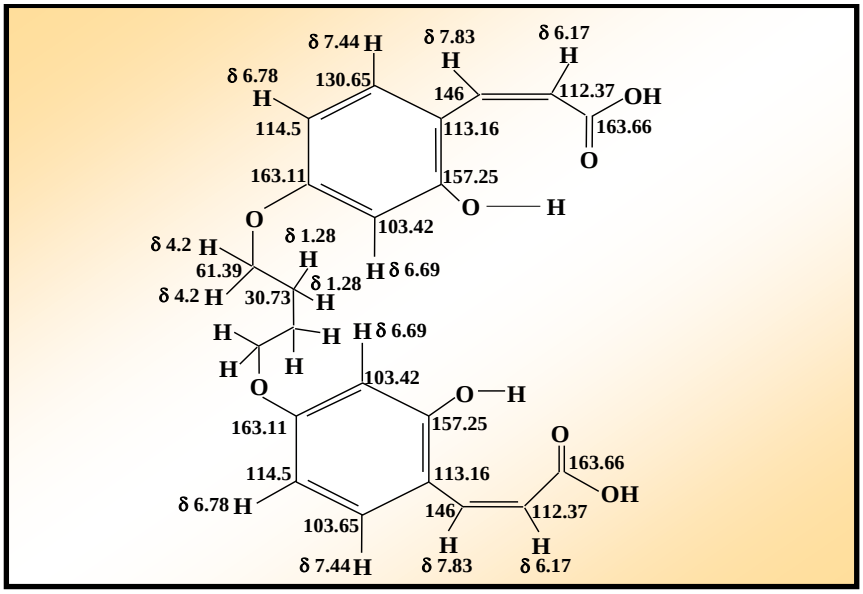
One factor which tend to lower the stretching frequency of carbonyl is due to the presence of conjugated system. However this effect will not decrease the frequency down to 1681.8 cm^{-1}. So there must be other factor that causes carboxylic acid carbonyl frequency. These unusual lower frequencies of phenolic – OH and carboxylic carbonyl group are assigned to be presence of intramolecular hydrogen bonding within the compound, as shown below.



Formation of intramolecular hydrogen bonding well accounts for observed lower frequencies of both –OH and C = O stretching vibration.In EI-Mass spectrum, the observation of the apparent peak at m/z 208 implies the following molecular structure.



However, the absence of methyl carbon in the DEPT spectrum and the occurrence of the molecular ion peak at m/z 414 indicates that the compound is suggested to exist as a dimer as described below.



Complete structure of the pure isolated compound

The IUPAC name of the pure compound isolated from the bark of *A. cordifolia* (Hnaw) is dimer of 2-hydroxy-4-ethoxy cis-coumaric acid.

Conclusion

In this research, the bark of *A. cordifolia* (Hnaw) was selected for chemical analysis. Green crystals (2.35 %) could be isolated from ethyl acetate crude extract in pure state. The molecular formula and the complete structure of the isolated compound were determined by some spectroscopic methods. This elucidated structure as dimer of 2-hydroxy-4-ethoxy cis-coumaric acid was then confirmed by mass fragmentation behaviours in EI-Mass spectral data. When antimcrobial activities of pure compound was determined, it exhibited medium activities against on *Bacillus pumilus* and *E.coli* and low activities against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*.

Acknowledgements

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