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Structure Elucidation and Antimicrobial Activities of the Isolated Compound (A) From the Ethyl Acetate Extract of Root of Curcuma wenyujin Y.H.Chen & C.Ling (Say Ta Lone)

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Abstract: In this paper, the Curcuma wenyujin Y.H.Chen & C.Ling Plant, one Myanmar medicinal plant was selected for chemical analysis. The roots of this plant were collected from Kani town ship, Monywa district, Sagaing region. The antimicrobial activities of the pure compound (A) that had been isolated from the ethyl acetate extract of Curcuma wenyujin Y.H.Chen & C.Ling root were determined by ager well diffusion method on the selected microorganisms. Moreover, the structure elucidation of the pure compound (A) was performed by FT-IR, HNMR, CNMR, DEFT, DQS- COSY, HSQC and HMBC respectively.

Keywords: Roots, Plant, Microorganisms, Antimicrobial Activities, Spectroscopic Methods.

I. INTRODUCTION

The plant kingdom is an effective source of natural bioactive compounds. These natural bioactive compounds can be obtained from the different parts of plants, such as roots, stems, fruits, flowers, and leaves etc. Alkaloids, steroids, flavonoids, terpenoids, glycosides, tannins, saponins, reducing sugars, phenolic compounds etc. are the plant derived chemicals called phytochemicals. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. [1] Herbal medicines have been proved to get genuine utility in the ancient and present time. Therefore, more and more people in the developing countries of the world are using the medicinal plants in treating and preventing their common diseases as the traditional medicines. The medicinal plants can play not only the need for humans' health services but also the need for their increased income and as a significant contribution to the national economics. In the world today pharmaceutical companies have the great interest in the phytochemical for the production of new potential drugs that can prevent the modern various diseases. Therefore, this medicinal plant ,Curcuma wenyujin Y.H.Chen & C. Ling belonging to the genus Curcuma, in the family Zingiberaceae was selected to study the antimicrobial activity and to elucidate the structure of pure compound (A) isolated from ethyl acetate extract of this plant.

II. DETERMINATION OF ANTIMICROBIAL ACTIVITY OF ISOLATED PURE COMPOUND (A)

The determination of antimicrobial activities was performed by ager well diffusion method using various solvents in Pharmaceutical research department (PRD), Insein Yangon. In this work, the tested strains of microorganisms were Bacillus Subtilis,Bacillus pumilus, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albican.

III. RESULTS AND DISCUSSION

A. Antimicrobial Activities of Pure Compound (A)

The antimicrobial activities of pure compound (A) were examined by using agar well diffusion method with six microorganisms. As the results, the compound (A) showed medium activity on the Bacillus subtilis, Bacillus pumilus, Candida albicans and E. coli, and low activity on the Staphylococcus aureus and Pseudomonas aeruginosa. These results are shown in Table 1.

Table1. The Antimicrobial Activities of Pure Compound(A)

No.	Types of Microorganism	Inhibition zone (mm)
1	Bacillus subtilis	15
2	Staphylococcus aureus	13
3	Pseudomonas aeruginosa	14
4	Bacillus pumilus	16
5	Candida albicans	15
6	E. coli	15

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

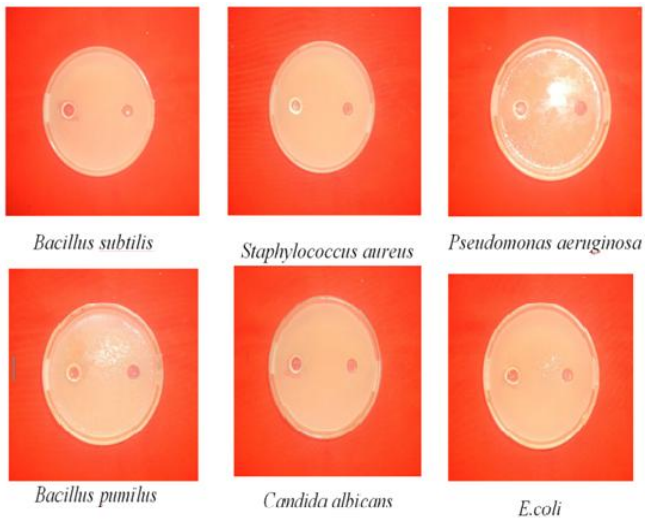
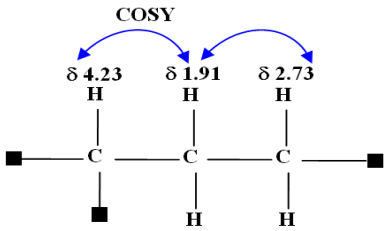


Fig 1: Antimicrobial Activities.

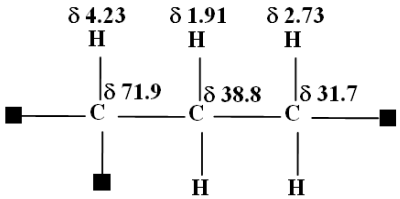
B. Structure elucidation of pure compound (A)

The structure elucidation of pure compound (A) was investigated by applying FT-IR, ^{1}HNMR, ^{13}CNMR, DEFT, DQS-COSY, HSQC and HMBC respectively. In DQF-COSY spectrum, the two sp^3 methylene protons (δ 2.73ppm and 1.91 ppm) and one sp^3 methine proton (δ 4.23 ppm) directly correlated with each other giving rise to the following fragment (1).



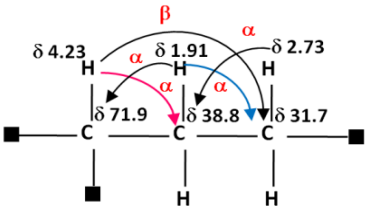
Fragment (1)

In HSQC spectrum, these two sp^3 methylene protons (δ 2.73 ppm and 1.91 ppm) and the sp^3 methine proton (δ 4.23 ppm) directly attached to sp^3 methylene carbons (δ 31.7ppm and 38.8 ppm) and sp^3 methine carbon (δ 71.9 ppm) as shown below.



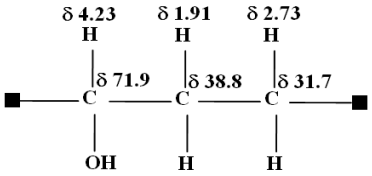
Fragment (1)

The existence of this fragment could be confirmed by α ^{1}H - ^{13}C long range signal between sp^3 methylene proton (δ 2.73 ppm) and sp^3 methylene carbon (δ 38.8 ppm) in HMBC spectrum. There was also the α ^{1}H - ^{13}C long range coupling of sp^3 methylene proton (δ 1.91 ppm) with sp^3 methylene carbon (δ 31.7 ppm) and sp^3 methine carbon (δ 71.9 ppm). Moreover, sp^3 methine proton (δ 4.23ppm) had α and β ^{1}H - ^{13}C long range coupling of sp^3 methylene carbon (δ 38.8 ppm) and (δ 31.7 ppm).



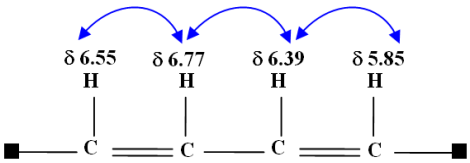
Fragment (1)

In the DEPT spectrum, sp^3 methine carbon which appeared at δ 71.9 ppm should be carbinol carbon attached by only one proton. Hence, this alcohol should be secondary alcohol as indicated below.



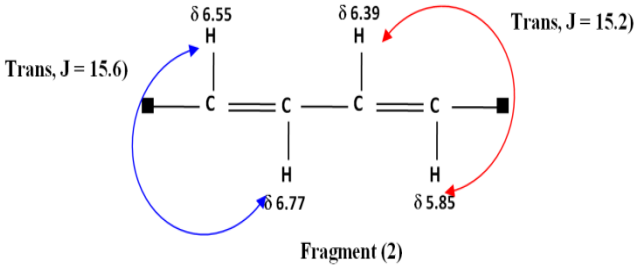
Fragment (1)

The formation of – OH functional group could also be confirmed by FT-IR spectrum. This spectrum showed -OH stretching vibration band at 3355.29 cm^{-1}, C – C – O stretching vibration band of alcohol at 1176.62 cm^{-1} and -OH out of plane bending vibration of alcohol group at 695.36 cm^{-1}. Furthermore, the DQF-COSY spectrum, showed the ^{1}H-^{1}H coupling of the sp^2 methine protons (δ 5.85 ppm, δ 6.39 ppm, δ 6.77 ppm and δ 6.55 ppm) which gave rise to the following fragment (2).



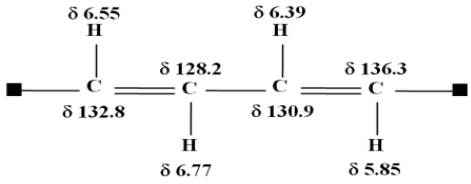
Fragment (2)

On the other hand, in ^{1}H NMR spectrum, the splitting patterns and coupling constant (J values) of the two alkenic protons (δ 5.85 ppm, dd, J = 6.8 Hz, 15.2 Hz) and (δ 6.39 ppm, dd, J = 10.5 Hz, 15.3 Hz) implied that these protons must be in trans position. Similarly, since the splitting patterns and coupling constant (J values) of the two alkenic protons were (δ 6.77 ppm, dd, J = 10.5 Hz, 15.6 Hz) and (δ 6.55 ppm, d, J = 15.7 Hz) , these protons were oriented as trans alkenic to each other in fragment (2)



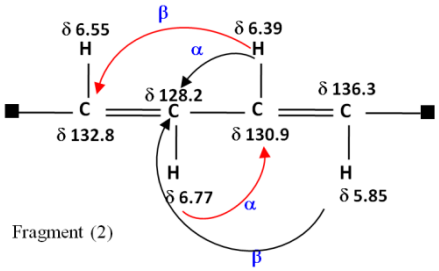
Fragment (2)

According to the HSQC spectrum, these alkenic protons directly attached to their respective alkenic carbons as follows.



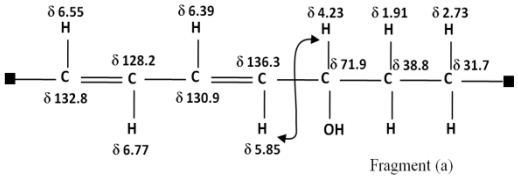
Fragment (2)

The observation of α and β ^{1}H -^{13}C long range signals of the alkenic protons (δ 6.39 ppm and δ 5.85 ppm) with sp^2 methine carbon (δ 128.2 ppm) indicated the evidence for this fragment (2) in HMBC spectrum. Moreover, sp^2 methine proton (δ 6.39 ppm) has β ^{1}H -^{13}C long range coupling with sp^2 methine carbon (δ 132.8 ppm) and sp^2 methine proton (δ 6.77 ppm) had α ^{1}H -^{13}C long range correlation with sp^2 methine carbon (δ 130.9 ppm)



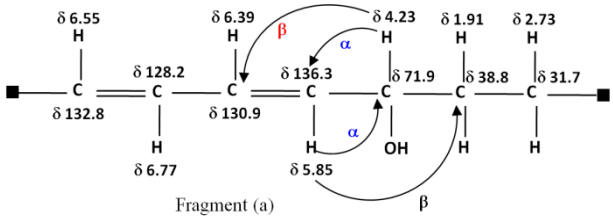
Fragment (2)

This fragment (2) could be connected with the fragment (1) due to the ^{1}H -^{1}H coupling of sp^3 methine proton (δ 4.23 ppm) and of sp^2 methine proton (δ 5.85 ppm) in DQS-COSY spectrum, yielding to the following extended fragment (a).



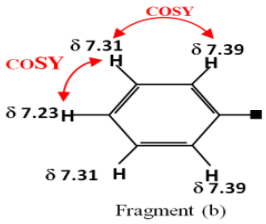
Fragment (a)

This connection could be confirmed by α and β ^{1}H -^{13}C long range signals of the sp^3 methine proton (δ 4.23 ppm) with sp^2 methine carbon (δ 136.3 ppm) and other sp^2 methine carbon (δ 130.9 ppm). In addition, sp^2 methine proton (δ 5.85 ppm) had α and β ^{1}H -^{13}C long range coupling with sp^3 methine carbon (δ71.9 ppm) and the sp^3 methylene carbon (δ 38.8 ppm) in HMBC spectrum.



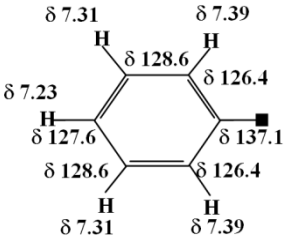
Fragment (a)

In DQF-COSY spectrum, the two equivalent protons (δ 7.31 ppm) directly coupled to another equivalent proton (δ 7.39 ppm) and also coupled to aromatic proton (δ 7.23 ppm) giving the following mono substituted benzene ring fragment (b).



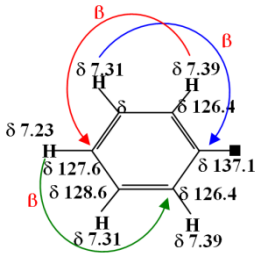
Fragment (b)

The HSQC spectrum, supported ^{1}H -^{13}C direct correlation of the aromatic protons (δ 7.23 ppm, δ 7.31 ppm and δ 7.39 ppm) with their respective aromatic carbons (δ 127.6 ppm, δ 128.6 ppm and δ 126.4 ppm) as follows.



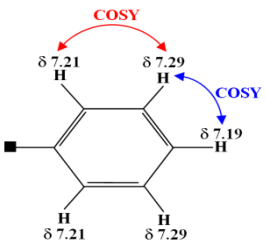
Fragment (b)

In HMBC spectrum, the sp^2 methine proton (δ 7.31 ppm) has β long range coupling with sp^2 quaternary carbon (δ 137.1 ppm). Moreover, the ^{1}H -^{13}C long range coupling between sp^2 methine proton (δ 7.39 ppm) and the aromatic carbon (δ 127.6 ppm) and between the aromatic proton (δ 7.23 ppm) and the aromatic carbon (δ 126.4 ppm) could also be observed as indicated below.



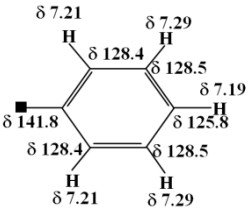
Fragment (b)

The two equivalent sp^2 methine protons (δ 7.29 ppm) were found to be coupled to another sp^2 methine protons (δ 7.19 ppm) and (δ 7.21 ppm) in the DQF-COSY spectrum, yielding the following mono substituted benzene ring fragment (c).



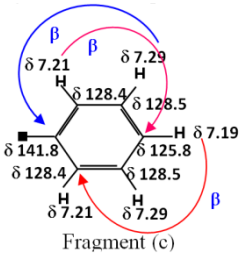
Fragment (c)

According to the HSQC spectrum, the sp^2 methine protons (δ 7.19 ppm, δ 7.29 ppm and δ 7.21 ppm) directly connected to the corresponding aromatic carbons (δ 125.8 ppm, δ 128.5 ppm and δ 128.4 ppm) as below fragment (c).



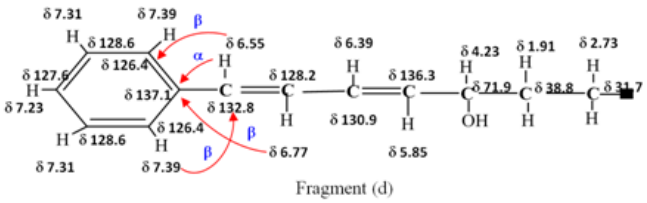
Fragment (c)

The sp^3 methine proton (δ 7.21 ppm) has β ^1H-^{13}C long range correlation with sp^2 methine carbon (δ 125.8 ppm). Furthermore, the β ^1H-^{13}C long range correlation between the aromatic proton (δ 7.29 ppm) and sp^2 quaternary carbon (δ 141.8 ppm) and between the sp^2 methine proton (δ 7.19 ppm) and the aromatic carbon (δ 128.4 ppm) could be determined by the HMBC spectrum, and gave rise to the following fragment (c).



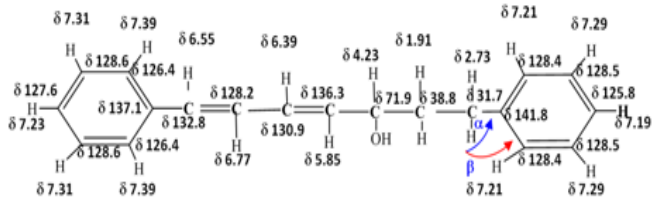
Fragment (c)

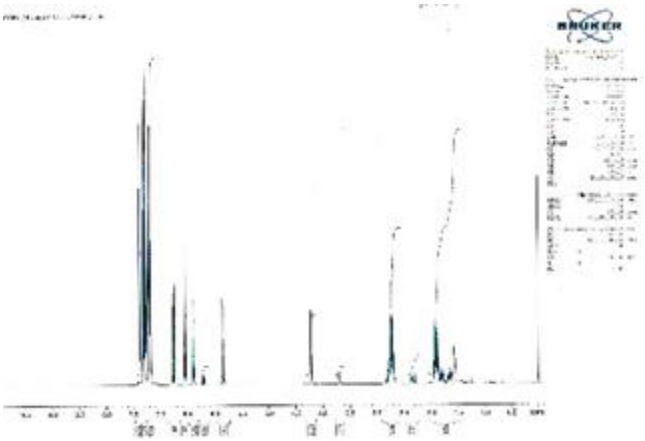
According to HMBC spectrum, the fragment (a) and the fragment (b) could be correlated due to the α and β -^1H-^{13}C long range coupling of sp^2 methine proton (δ 6.55 ppm) from fragment (a) with the aromatic sp^2 quaternary carbon (δ 137.1 ppm) and aromatic methine carbon (δ 126.4 ppm) from fragment (b). Moreover, there was also the β ^1H-^{13}C long range correlation between sp^2 methine proton (δ 6.77 ppm) from fragment (a) and the aromatic quaternary carbon (δ 137.1 ppm) from fragment (b).Then, the aromatic proton (δ 7.39 ppm) from fragment (b) has β ^1H-^{13}C long range coupling with sp^2 methine carbon (δ 132.8 ppm) from fragment (a) .These correlations gave rise to the following extended fragment (d).



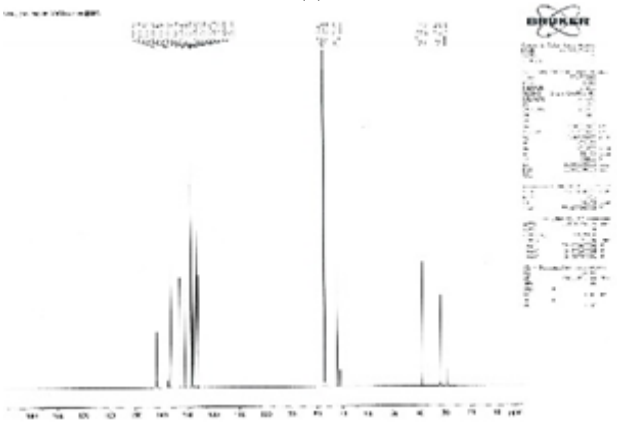
Fragment (d)

Furthermore, this fragment (d) could be connected with the former fragment (c) in HMBC spectrum, by the observation of α and β ^1H-^{13}C long range coupling of the sp^2 methine proton (δ 2.73 ppm) with the aromatic quaternary carbon (δ 141.8 ppm) and the aromatic carbon (δ 128.4 ppm) respectively leading to the following complete structure of isolated pure compound(A).This pure compound (A) is (1E 3E) hepta -1, 3-diene 1,7diyl) dibenzene.

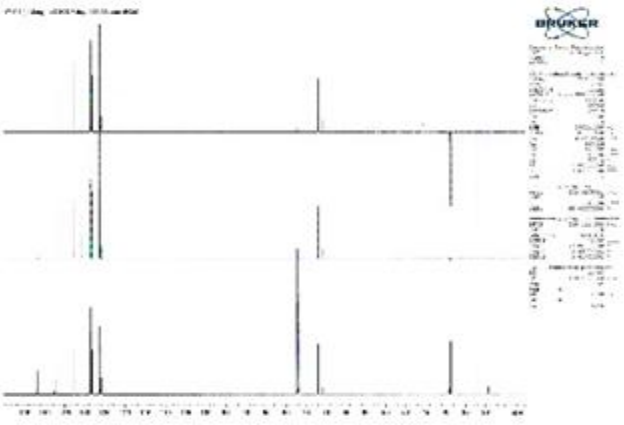




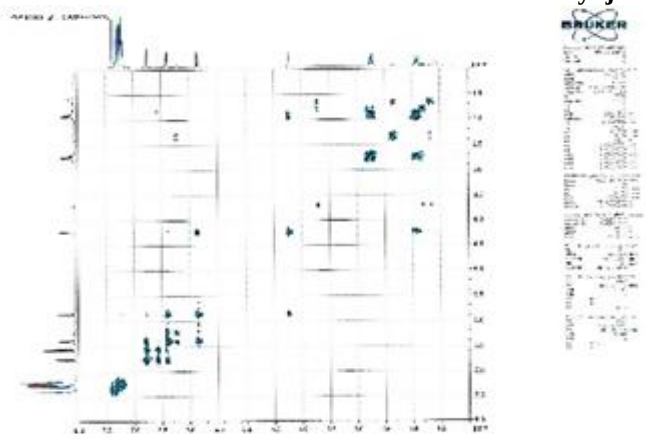
(a)



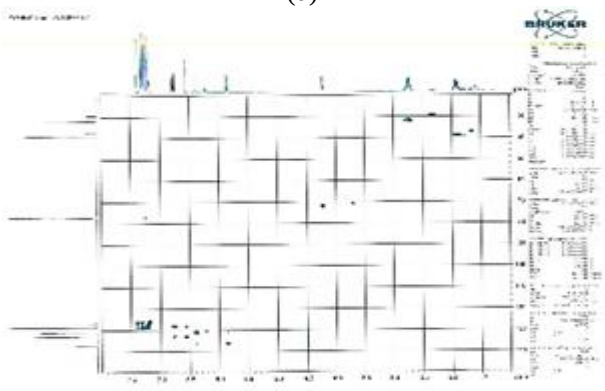
(b)



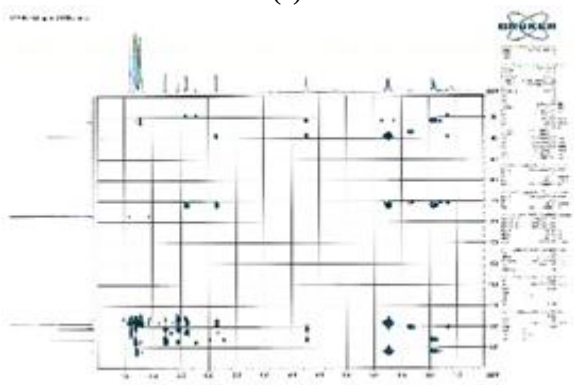
(c)



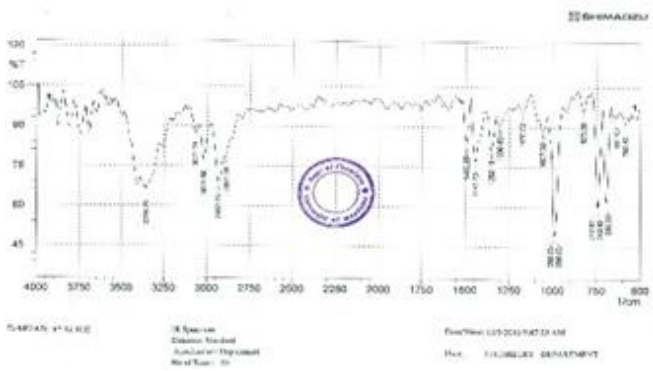
(d)



(e)



(f)



(g)

Fig2. (a) HNMR, (b) CNMR, (c) DEPT, (d) DQS-COSY, (e) HSQC , (f) HMBC, (g) FT-IR.

IV. CONCLUSION

In this research work, one important Myanmar medicinal plant, Curcuma wenyujin Y.H. Chen & C. Ling was collected for chemical analysis. According to the antimicrobial activity test, the pure compound (A) isolated from ethyl acetate extract of the Curcuma wenyujin Y.H.Chen & C.Ling root had the medium activity on the Bacillus subtilis, Bacillus pumilus, Candida albicans and E. coli and low activity on the Staphylococcus aureus and Pseudomonas aeruginosa. Moreover, the structure elucidation of pure compound (A) was performed by some spectroscopic techniques. The name of this pure compound (A) is (1E 3E) hepta - 1,3-diene 1,7diyl) dibenzene.

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