ISOLATION OF STIGMASTEROL FROM METHANOLIC EXTRACT OF STEM AND FOLIAGE OF *ABRUS PRECATORIUS* LINN

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Abstract - *Abrus precatorius* Linn. belongs to Fabaceae family. Its aerial parts are used for treating certain health problems like leucorrhoea, gonorrhoea, diarrhoea and dysentery. The purpose of this study is to identify, isolate and determine structure of isolated compound from the stem and foliage of red form of *Abrus precatorius* Linn which are collected from the Dai Loc District, Quang Nam Province, Vietnam. To isolate the compound, the dried stem and foliage powder of *Abrus precatorius* Linn are extracted with methanol at room temperature and the solvent is recovered under low pressure to obtain crude methanol extract. Crude methanol extract is extracted again with dichloromethane and the solvent is recovered under low pressure to obtain crude dichloromethane extract. The isolated compound is purified from crude dichloromethane extract by silica gel column chromatography. The structure of the isolated compound is determined as Stigmasterol by IR, ^{1}H-NMR, ^{13}C-NMR and GC.

Key words - *Abrus precatorius* Linn; Stigmasterol; compound; isolation; NMR.

1. Introduction

Abrus precatorius Linn. belongs to family Fabaceae. Its aerial parts are used for treating certain health problems like leucorrhoea, gonorrhoea, diarrhoea and dysentery. The roots, leaves and seeds are used for medicinal purpose. General phytochemical screening of *Abrus precatorius* Linn. reveals the presence of steroids, alkaloids, saponins, phenolic compounds, terpenes. (20S,22S)-3beta,22-dihydroxycucurbita-5(10),24-dien-26,29-dioic acid delta-lacton; 3-O-[6'-methyl-beta-D-glucuronopyranosyl]-3beta, 22beta-dihydroxyolean-12-en-29-oic acid methyl ester ; 3-O-beta-D-glucuronopyranosylsophoradiol methyl ester isolated from the foliage of *Abrus precatorius*[1]. Some bioactive compounds such as 3-O-β-D-glucopyranosyl-(1→2)- β-D-glucopyranosyl subprogenin D, subprogenin D, abrusgenic acid, triptotriterpenic acid B, abruslactone A, abrusogenin and abrusoside C are also isolated and purified from leaves and stem of this plant[2].

2. Experimental

2.1. Instruments and Chemicals

The stem and foliage of red form of *Abrus precatorius* Linn were collected from the Dailoc District, Quangnam Province, Vietnam in March, 2014. The plant was identified and confirmed by Master Nguyen Thi Đao, Department of Biology & Environmental Science, University of Education - University of Danang ,Vietnam. The stem and foliage of *Abrus precatorius* Linn. are manually separated then air dried, powdered, sieved, weighed and stored in airtight containers and subsequently referred to as powdered drug.

2.2. Extraction and Isolation method

Powder (1.0 kg) of stem and foliage of red form of *Abrus precatorius* Linn are immerged in methanol (2.5litres) for 15 days at room temperature and then solvent is recovered under pressure to obtain 50 g crude methanol extract. Crude methanol extract is dissolved with distilled water, extracted again with dichloromethane and then solvent is recovered under pressure to obtain 10 g crude dichloromethane extract.

A small portion of crude dichloromethane extracted is dissolved in dichloromethane and the solution is spotted on TLC plates. Then the TLC plates are run by specific solvent system and are viewed individually under UV light and also with the H_2SO_4 10% reagent.

A portion of dichloromethane extract (2 g) is subjected to column chromatography (silica gel, 2x70 cm). The column is eluted with the solvent system of n-hexane - ethyl acetate (starting with the proportion of 9:1 and finishing with the proportion of 8:2) give 4 fractions: CTD01 (78 mg), CTD02 (244 mg), CTD03 (67 mg) and CTD04. Fraction CTD02 (244 mg) is subjected again to column chromatography (silica gel, 0.5x100 cm) to give 4 compounds: CTD02.1 (83 mg), CTD02.2 (30 mg), CTD02.3 (10 mg) and CTD02.4.

2.3. Spectroscopic characterization

Different spectroscopic methods are used to elucidate the structure of isolated compound CTD02.2. Among the spectroscopic techniques GC/MS, IR, ^{1}H-NMR, ^{13}C-NMR are carried out.

GC spectra is recorded on Agilent 7890A/5975C; IR spectrum is recorded on NICOLETiS-10, ^{1}H-NMR and ^{13}C-NMR spectra are recorded on Bruker Advance. The ^{1}H-NMR spectra is recorded at 500MHz and the ^{13}C-NMR spectra is recorded at 125 MHz using $CDCl_3$ as solvent with TMS as an internal standard.

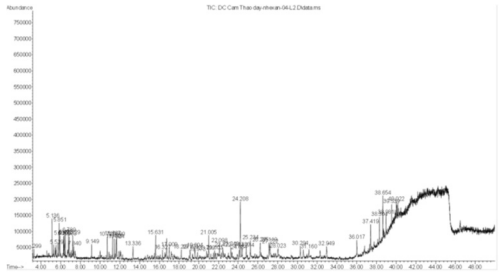

Figure 1. *Chromatogram of CTD01*

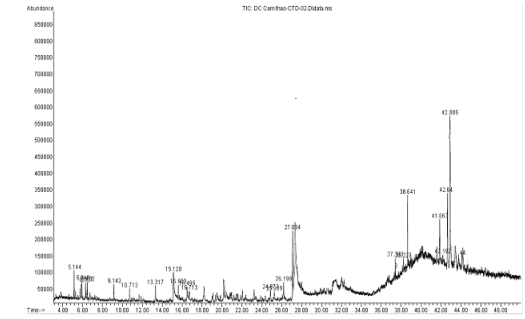

Figure 2. *Chromatogram of CTD02*

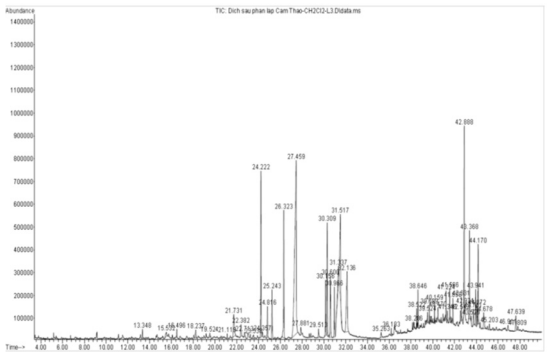

Figure 3. *Chromatogram of CTD03*

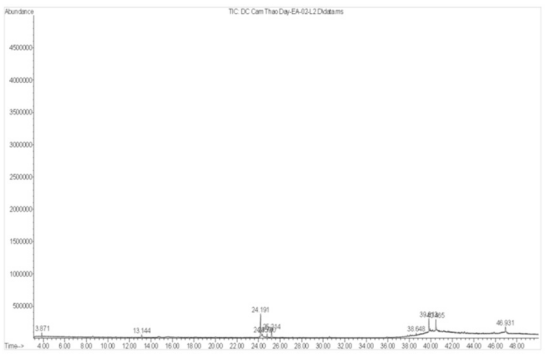

Figure 4. *Chromatogram of CTD04*

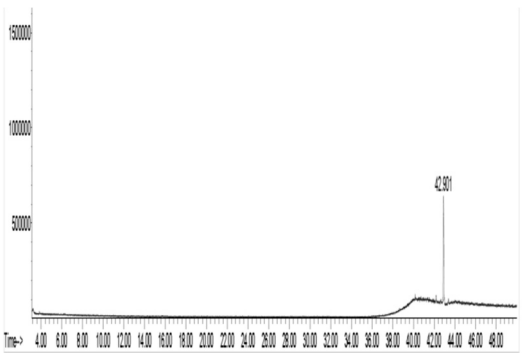

Figure 5. *Chromatogram of CTD02.2*

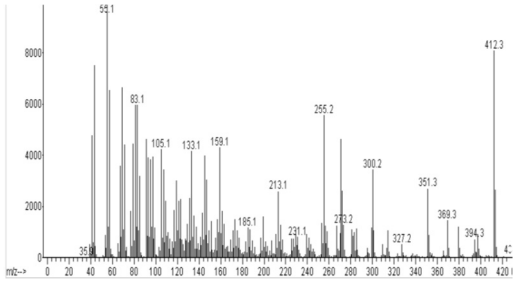

Figure 6. *Mass Spectra of CTD02.2*

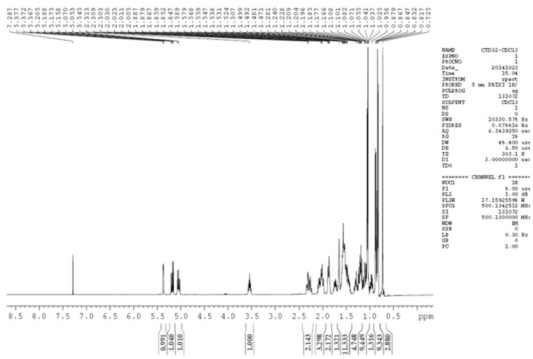

Figure 7. *^{1}H-NMR spectroscopy of CTD02.2*

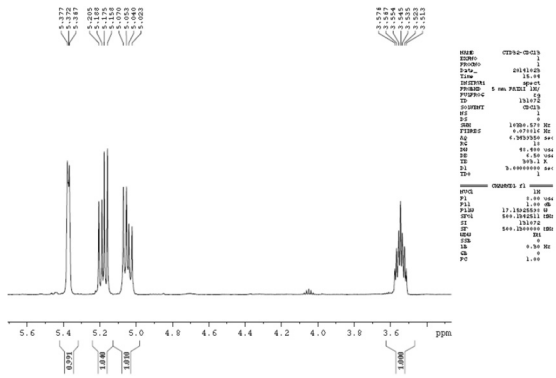

Figure 8. *^{1}H-NMR spectroscopy of CTD02.2 (δ 3-6ppm)*

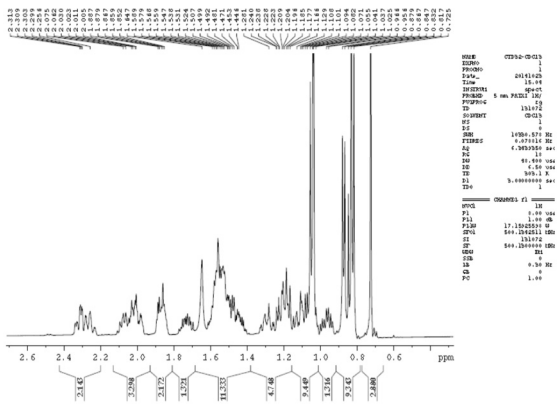

Figure 9. *^{1}H-NMR spectroscopy of CTD02.2 (δ 0 - 3 ppm)*

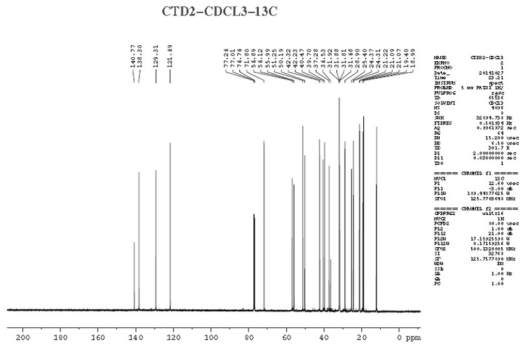

Figure 10. *^{13}C-NMR spectroscopy of CTD02.2*

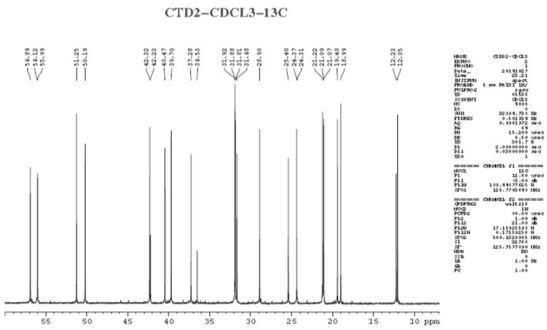

Figure 11. *^{13}C-NMR spectroscopy of CTD02.2 (δ 10-60 ppm)*

3. Results and Discussion

The CTD02.2 is a white solid crystal.

MS of CTD02.2: m/z base peak 412.3(M+, $C_{29}H_{48}O$), 394.3, 369.3, 351.3, 327.2, 300.2, 273.2, 255.2, 231.1, 213.1, 199.4, 185.1, 159.1, 145.6, 133.1, 121.2, 105.1, 91.4, 83.1, 69.7, 55.1, 41.5.

The IR(KBr) absorption spectrum shows absorption peaks at 3422.15cm^{-1}, 2937.44cm^{-1} and 2867.32cm^{-1}, 1654.04cm^{-1}, 1636.75 cm^{-1}, 1382.12cm^{-1}, 1192.60cm^{-1}, 1051.86cm^{-1}, 970.45cm^{-1}, 799.83cm^{-1}, 591.93cm^{-1} and 419.35cm^{-1}.

^{1}H-NMR (500 MHz, $CDCl_3$) of CTD02.2: ^{1}H-NMR has given signals at δ 7.287, 5.377, 5.372, 5.367, 5.205, 5.188, 5.175, 5.158, 5.070, 5.053, 3.545, 2.313, 2.309, 2.303, 2.030, 2.023, 2.011, 2.005, 1.887, 1.879, 1.867, 1.859, 1.852, 1.647, 1.589, 1.579, 1.568, 1.559, 1.547, 1.538, 1.531, 1.524, 1.507, 1.499, 1.492, 1.481, 1.471, 1.281, 1.240, 1.228, 1.209, 1.204, 1.196, 1.185, 1.177, 1.166, 1.108, 1.101, 1.082, 1.071, 1.055, 1.042, 1.037, 1.025, 0.956, 0.879, 0.867, 0.847, 0.832, 0.817, 0.725.

^{13}C-NMR (125 MHz, $CDCl_3$) of CTD02.2: ^{13}C-NMR has given signals at 37.28 (C-1), 31.88 (C-2), 71.80(C-3), 42.23(C-4), 140.77(C-5), 121.69(C-6), 31.92(C-7), 31.88(C-8), 50.19(C-9), 36.53(C-10), 21.06(C-11), 39.70(C-12), 42.23(C-13), 56.89(C-14), 24.37(C-15), 28.90(C-16), 55.99(C-17), 12.23(C-18), 21.07(C-19), 40.47(C-20), 21.22(C-21), 138.30(C-22), 129.31(C-23), 51.24(C-24), 31.88(C-25), 18.99(C-26), 19.40(C-27), 25.40(C-28), 12.05(C-29).

The MS spectrum shows a parent molecular ion $[M+H]^+$ peak at m/z 412.3 which correspond to the molecular formula $C_{29}H_{48}O$.

The IR signal absorption band observed at 3422.15 cm^{-1} is characteristic of O-H stretching. Absorption at 2937.44 cm^{-1} and 2867.32 cm^{-1} is due to aliphatic C-H stretching. Other absorptions at 1636.75 - 1654.06 cm^{-1} are because of C=C stretching, however this band is weak. Absorption at 1458.46 cm^{-1} is a bending frequency for cyclic $(CH_2)_n$. Absorption at 1382.12cm^{-1} is attributable to OH deforming absorption. The absorption frequency at 1051.86 cm^{-1} signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol.

Similarly, from ^{1}H-NMR data of CTD02.2, it is seen that carbinylic proton appears at δ 3.51 (1H, m, H-3). Three vinylic protons appears at δ 5.37 (1H, d, J = 5Hz, H-6), δ 5.02 (1H, dd, J = 15.0Hz and 8.5Hz, H-22), δ 5.16 (1H, dd, J = 15.0Hz and 8.5Hz, H-23). Six methyl protons also appears at δ 0.73 (3H, s, H-18), δ 1.06 (3H, s, H-19); δ 0.81 (3H, d, J= 7.5Hz, H-27), δ 0.86 (3H, d, J= 7.5Hz, H-26) và δ 0.82 (3H, d, J= 7.5Hz, H-21); δ = 0.84 (3H, t, H-29) [3].

The ^{13}C-NMR of CTD02.2 shows a total of 29 carbons. Signals δ 140.77 ppm and 121.69 ppm are assignable to the double bond at C_5 and C_6 [4]. The alkene carbons appear at 138.30 ppm (C_{22}) and 129.31ppm (C_{23}) [5]. The δ value observed at 71.80 ppm is due to C_3 hydroxyl group[6]. The value at δ 12.23 ppm and 19.40 ppm corresponds to angular carbon atoms (C_{18} and C_{19}). Spectra shows 29 carbon signals including six methyls, nine methylenes, eleven methanes and three quaternary carbons.

Table 1. *^{1}H-NMR and ^{13}C-NMR data of compound CTD02.2 and Stigmasterol.*

STT	Compound CTD 02.2		Stigmasterol [3]	
C	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)
1	37.28		37.28	
2	31.88		31.67	
3	71.80	3.50 (1H, m, H-3)	71.78	3.49 (1H, m, H-3)
4	42.23		42.32	
5	140.77		140.76	
6	121.69	5.37 (1H, d, J = 5,2 Hz, H-6)	121.66	5.33 (1H, d, J = 5.2 Hz, H-6)
7	31.92		31.92	
8	31.88		31.90	
9	50.19		50.20	
10	36.53		36. 52	
11	21.06		21.08	
12	39.70		39.70	
13	42.23		42.23	
14	56.89		56.88	
15	24.37		24.36	
16	28.90		28.89	

17	55.99		55.99	
18	12.23	0.73 (3H, s, H-18)	12.22	0.69 (3H, s, H-18)
19	21.07	1.06 (3H, s, H-19)	21.06	1.01 (3H, s, H-19)
20	40.47		40.50	
21	21.22	0.96 (3H, d, J= 7.5Hz, H-21)	21.22	0.92 (3H, d, J=6.5Hz, H-21)
22	138.30	5.05 (1H, dd, J = 8.5Hz and 15.0Hz, H-22)	138.28	5.03 (1H, dd, J=8.5Hz, 15.5Hz, H-22)
23	129.31	δ = 5.16 (dd, J = 8.5Hz and 15.0 Hz, H-23)	129.30	5.11 (1H, J=15.5Hz, 8.5Hz, H-23)
24	51.24		51.25	
25	31.88		31.87	
26	18.99	0.85 (3H, d, J= 7.5Hz, H-26)	18.99	0.84 (3H, d, J=6.5Hz, H-26)
27	19.40	0.81 (3H, d, J= 7.5Hz, H-27)	19.39	0.81 (3H, d, J=6.5Hz, H-27)
28	25.4		25.39	
29	12.05	0.82 (3H, d, J= 7.5Hz, H-29)	12.05	

4. Conclusion

We have isolated the compound CTD02.2 from the stem and foliage of red form of *Abrus precatorius* Linn collected from the Dai Loc District, Quang Nam Province, Vietnam. The isolated compound is purified by silica gel column chromatography. From the above IR, ^{1}H-NMR, ^{13}C-NMR and MS spectral data with the comparison made so far, it is concluded that sample CTD02.2 is Stigmasterol (Figure 5).

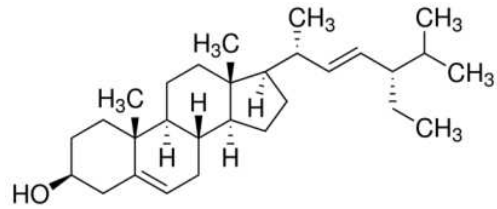

Figure 5. *Chemical structure of CTD02.2 (Stigmasterol)*

REFERENCES

[1] Nam-Cheol Kim, Darrick Kim & A. Douglas Kinghorn. New triterpenoids from the leaves of *Abrus precatorius* Linn. *Natural Product Letters*. 2002; 4: 261-266.

[2] Xiao, Zhi-Hui; Wang, Fa-Zuo; Sun, Ai-Jun; Li, Chuan-Rong; Huang, Cai-Guo; Zhang, Si. A New Triterpenoid Saponin from *Abrus precatorius* Linn. *Molecules*. 2012; 17: 295-302.

[3] Yinusa ISAH, IlogbulemG NDUKWE, Joseph O AMUPITAN. Isolation of Stigmasterol from aerial plant part of *Spillanthes Acmella* Murr. *World J Life Sci. and Medical* Research 2012; 2(2):77-81.

[4] Agrawal PK., Jain DC., Gupta RK., and Thakur RS. Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. *Phytochemistry*. 1985; 24(11): 2476-2496.

[5] Pretsch EB., Affolter A. Structure determination of organic compounds Table of spectra data. *Springer-Verlag*. Berlin Heidelberg. 2000; P.71-150.

[6] Jamal AK., Yaacob WA., and Din LB. A Chemical study on Phyllanthus Columnaris. *European Juornal of Scientific Research*. 2009; 28(1): 76-81.

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