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Der Pharma Chemica, 2011, 3 (6):160-164
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Chemical Examination of Fruits of *Cleistanthus collinus*

[1]J. Chandravathi Devi and [2]K. Vasantha Kumar Pai*

[1]Department of Chemistry, Rayalaseema University, Kurnool, AP, India
[2]Department of Industrial Chemistry, Kuvempu University, Shankarghatta, Karnataka, India

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ABSTRACT

The Cleistanthus Collinus have been examined for their chemical constituents having antibacterial and antifungal activity.

Keywords: Cleistanthus Collinus, soxlet apparatus, molisch test, diphyllin.

INTRODUCTION

Several species of the genus Cleistanthus have been chemically examined. The bark, leaves, heartwood, fruits and other parts of this plant are rich in lignins and lignin lactones. These lignin lactones are structurally similar to the semi-synthetic podophyllotoxin[1-4] anticancer drugs such as etopoide[5-10] and tinoposide. *Cleistanthus Collinus* has created a considerable amount of interest in recent years because of its complex metabolites and their cytotoxic activities. The other compounds isolated from the plants of this genus in addition to the lignins were terpenoids, lignin coumarins, flavones, alkaloids, steroids, alkamides and coumarino lignoids[11-15].

MATERIALS AND METHODS

Experimental section:
Cleistanthus collinus fruits were collected from Ethurunagaram Forest, Near Warangal, Andhra Pradesh, India. The fruit powder 5kg was extracted with petroleum ether (5 lit X 3 times) in a soxhlet apparatus for 36 h. The petroleum ether extract on concentration yielded a green semi-solid, which was not worked out further. The defatted plant material was again extracted in a soxhlet apparatus with methanol (5 lit X 3 times) for 36 h, which on concentration yielded a brown semi-solid (20g). This was column chromatographed over silica gel (100-200 mesh) and eluted as Ethyl acetate, Petroleum ether fractions 1-25 (250mL each) and petroleum ether: chloroform, chloroform: ethy1 acetate, ethy1 acetate. Petroleum ether fractions 1-25 (250ml each) and petroleum ether: chloroform fractions 26-75 on concentration yielded white waxy

solid, which was designated as CC-1 and identified as diphylin (1). Chloroform: ethyl acetate fractions 126-140 on concentration yielded white solid, which was designed as CC-III and identified as Cleistanthoside- A (3). The chloroform: ethyl acetate fractions 141-175 on concentration yielded mixture of CC-III and CC-II. The ethyl acetate fractions 176- 195 on concentration yielded light brown solid, which was designed as CC-II and identified as 4-O-(3″O-methy1-β-D-glucopranosy1) diphyllin (2).

i. CC-I (diphyllin) (1):

Recrystallisation from methanol gave white solid (95mg) mp 292-294 ºC); IR (KBr); 3257 cm^{-1} (OH), 1714 cm^{-1} (γ-lactone, C=O), 1615 cm^{-1}, 1507 cm^{-1}, 1433 cm^{-1} (C=C) and 926 cm^{-1} (methlenedioxy group); UV (MeOH): 218 nm (log ε 4.68). 270nm (log ε 4.62), 293 nm (log ε 3.67), 325 nm (log ε 3.64), 260 nm (log ε 3.50); ^{1}H NMR ($CDC1_3$ + DMSO-d_6) (300MHz): δ 9.84 (s, 4-OH), 7.53 (s, H-8), 6.93 (s, H-5), 6.67-6.87 (m, H-2', 5', 6), 6.03 (d, J+1.5Hz, -OCH_2-), 5.26 (s, bezylic-CH_2O), 3.98 (7-OCH_3), 3.71 (6-OCH_3); ^{13}C NMR ($CDC1_3$ + DMSO-d6) (50.3 MHz): δ 169.6(C=O), 149.9 (C-6), 149.1(C-7), 146.5 (C-4'), 146.2 (C-3'), 144.4 (C-1), 129.6(C-9), 129.5 (C-10), 128.4(C-4), 123.0(C-3), 123.0 (C-6), 120.9 (C-2), 118.1(C-1), 110.3 (C-8), 107.1 (C-5), 105.2 (C-2), 100.2 (C-5), 100.2 (-OCH_2-), 66.1 (benzylic-CH_2O), 55.2 (C-6-OCH_3), 54.8 (C-7-OCH_3); FABMS: m/z 381 $[M+H]^+$.

ii. CC-II (4-O-(3″-O-methy1-β-D-glucopyranosy1)diphyllin) (2):

Recrystallisation from chloroform gave light brown colored crystals mp 153-155 °C; IR (KBr): 3458 cm^{-1} (OH), 1762cm $^{-1}$ (C=O), 1623 $^{-1}$, 1512 cm^{-1}, 1512 cm^{-1}, 1437cm^{-1} (aromatic C=C) and 934 cm^{-1} (-OCH_2O); UV (MeOH): 314 nm (log ε 3.97), 297nm (log ε 3.71), 260nm (log ε 4.64) and 228nm (log ε 4.76); ^{1}H NMR ($CDC1_3$ + DMSO-d6) (200 MHz): δ 8.07 (s, H-5), 6.93 (s, H-8), 6.65-6.89 (m, H-2',5',6'), 6.03(d, J=1.5Hz, OCH_2O), 5.49 (AB Quartet, benzylic methylene), 4.62 (d, J=6.0 Hz, anomeric sugar monomer proton H-1″), 3.96 (s,6-OCH_3), 3.72(s,7-OCH_3), 3.60 (s, sugar 3″-OCH_3), 2.95-3.50 (m, other sugar protons); ^{13}C NMR ($CDC1_3$ + DMSO-d6) (100.6 MHz): δ169.7 (C=O), 151.6 (C-7), 150.1 (C-6), 147.2 (C-3'), 147.2 (C-4'), 143.8 (C-1), 135.0 (C-10), 130.2 (C-9), 128.4 (C-4), 128.3 (C-3), 126.6 (C-2), 123.5(C-6'), 118.9 (C-1), 110.7(C-8), 107.9 (C-5'), 105.7 (C-2'), 103.7(C-5), 101.6(-OCH_2O-), 101.2 (anomeric sugar carbon C-1), 78.1 (C-3″), 74.2 (C-5''), 70.2 (C-4″), 67.4(benzylic-CH_2O). 61.5 (C-2″), 59.7(C-6″), 57.7 (C-3″). 74.2 (C-5″), 70.2 (C-4), 67.4 (benzylic-CH_2O), 61.5(C-2″), 59.7 (C-6″), 57.7 (C-3″-OCH_3), 56.3(C-6-OCH_3) and 55.5 (C-7-OCH_3); FABMS: m/z 557 $[M+H]^+$, 381 (M-sugar + 2H), 425, 403, 301, 277, 200, 137 and 115.

3. CC-III (Cleistanthoside-A) (3):

Recrystallisation from choloroform: acetone mixture as white colored solid mp 238-240 °C IR (KBr): 3409 cm^{-1} (OH), 1754 cm^{-1} (C=O), 1526 cm^{-1} (aromatic C=C) and 927 cm^{-1} (OCH_2O). UV (MeOH): 322 nm (log ε 3.75), 298 nm (log ε 3.62) and 263 nm (log ε 4.36); ^{1}H NMR ($CDC1_3$ + DMSO-d6) (300 MHz): 7.84 (s, H-5), 6.99 (s, H-8) 6.68-6.96 (m, H-2',5',6'), 6.08 (d, J=1.5Hz, OCH_2O), 5.50 (AB quartet, benzylic methylene), 5.14 (d, J=6.0 Hz, anomeric glucopyranose proton H-1″), 4.71 (d, J=6.0 Hz, anomeric xylopyranose proton H-1″), 4.02 (s, 6-OCH_3) and 3.07-3.49 (m, other two sugars protons); ^{13}C NMR ($CDC1_3$ + DMSO-d6) (100.3 MHz): 169.5 (C=O), 151.5 (C-7), 150.0 (C-6), 147.2 (C-3') 147.1(C-4') 143.8(C-1), 134.9 (C-10), 130.1 (C-9), 128.3 (C-4), 128.3 (C-3), 126.5 (C-2), 123.3 (C-6), 118.8 (C-1), 110.5(C-8), 107.8 (C-5'), 105.6(C-2), 103.7 (C-5), 101.7 (-OCH_2O), 101.3 (anomeric xylopyranose carbon

C-1″), 101.1 (anomeric glucopyranose carbon C-1″), 81.9 (C-3″), 77.1(C-2″), 76.6 (C-4), 74.1 (C-5″), 70.1(C-4), 67.2 (benzylic-CH_2O), 61.4 (C-2), 61.3(C-5), 59.6(C-6″), 57.6 (C-3″-OCH_3), 56.2 (C-4″-OCH_3), 55.7(C-6-OCH_3) and 55.4(C-7-OCH_3); FABMS: m/z 703 $[M+H]^+$, 725 $[M+Na]^+$, 541 [M-1xsugar+2H], 381[M-2xsugar+2H], 351,321,293,185,161,143 and 129.

RESULTS AND DISCUSSION

The fruit powder of *Cleistanthus Collinus* was extracted with petroleum ether using soxhlet. The petroleum ether extract on concentration yielded green semi-solid containing fatty acids, which was kept aside and not worked out further. The defatted dried powder was again extracted in a soxhlet apparatus with methanol. On concentration under vacuum yielded a brown semi-solid (20g).This was column chromatographed over silica gel (100-200 mesh) and eluted with increasing polarity of solvent systems. Three crystalline compounds **CC-I, CC-II** and **CC-III** were obtained.

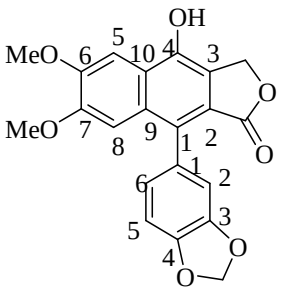
i. Structural elucidation of CC- I: *It is identified as diphyllin* ***(1):***

CC-I was crystallized from methanol as white solid, mp 292-294 ºC. It is analyzed for $C_{21}H_{16}O_7$ supported by the $[M+H]^+$ ion at m/z 381 from its FAB mass spectrum. It gave green color with methanolic ferric chloride indicating the presence of phenolic hydroxy1 (OH) group. CC-I was insoluble in 10% aq. Sodium bicarbonate, but was slowly soluble in dilute aq. sodium hydroxide giving a pale yellow color, indicating lactone ring system. It gave the negative Molisch test, showing it is not a glycoside**.** CC-I gave positive Labat test for methylenedioxy group.

The IR spectrum of CC-I showed broad peak at 3257 cm^{-1} which due to the phenolic OH group. The intense peak at 1714 cm^{-1} is assignable to γ-lactone carbony1. The peaks at 1615 cm^{-1}, 1507 cm^{-1} and 1433 cm^{-1} are due to aromatic rings. The intense peak at 926cm^{-1} is highly characteristic of the methylene dioxy group. The UV (MeOH) spectrum of CC-I showed absorptions at λ_{max} 218 nm (log ε 4.68), 270 nm (log ε 4.62), 293 nm (log ε 3.67), 325 nm (log ε 3.64) and 360 nm (log ε3.50).

The ^1H NMR spectrum of CC-I showed signals similar as earlier reported for *diphyllin.* The H-5 and H-8 appeared as singlet at δ 7.54 and δ 6.93 respectively. The two methoxy1s 6-OCH_3 and 7-OCH_3 appeared at δ 3.98 and 3.71. The three aromatic protons due to H-2', H-5' and H-6' appeared at δ 6.67-6.87 as multiplet. The methylene dioxy group at C-3', C-4' in the pendent ary1 ring appeared as a doublet at 6.03 (J=1.5 Hz) and benzylic methylene oxy group protons appeared as singlet at δ 5.26. The 4-OH appeared at δ 9.84 as a singlet.

The ^{13}C NMR spectrum of CC-I showed the lactone carbiny1 at δ 169.6. Six oxygenated aromatic carbons appeared δ 128.4 (C-4). 149.9(C-6), 149.1(C-7), 144.4 (C-1), 146.2 (C-3) and δ 146.5 (C-4). The other carbon signal assignments are at δ 118.1 (C-1), 120.9 (C-2), 123.0 (C-3), 129.6(C-9), 129.5 (C-10), 110.3 (C-8), 100.2 (C-5), 105.2 (C-2), 107.1 (C-5) and 123.0(C-6). The benzylic methylene appeared at 66.1, the methylenedioxy at 100.2 and the two methoxyls 7-OCH_3 and 6-OCH_3 appeared at δ 54.8 and 55.2.



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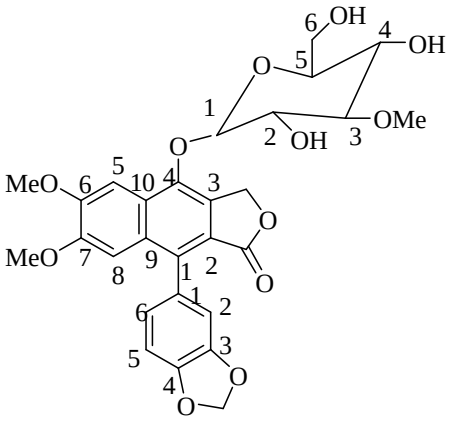
ii. Structural elucidation of CC-II: It is identified as 4-O-(*3″-O-methy1-β-D-glucopyranosyl) diphyllin* **(2).**



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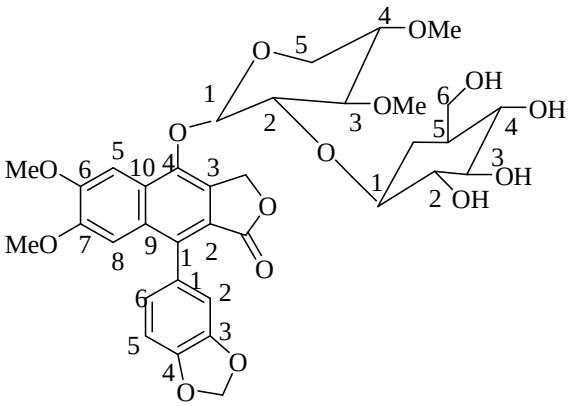
iii. Structural elucidation of CC-III: It is identified as Cleistanthoside-A **(3)**



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