PHYTOCHEMICAL INVESTIGATION OF BIOACTIVE CONSTITUENTS FROM STEM BARK OF *DICHROSTACHYS CINEREA*

[1]Syed Ayub, [2]Hurmath Jabeen,[3]Dr.G.krishnamohan,[4]Dr.K.Suresh Babu,[5]Dr.M.Sandhya Rani.
[1]B.pharm,[2]B.pharm [3]M.pharm ,Ph.D,[4]M.sc,Ph.D, PDF,[5]M.pharm,Ph.D.
[1]Pharmacognosy ,
[1,2,3,5]Jawaharlal Nehru Technological University,[4]Indian Institute of Chemical Technology, Hyderabad, India.

Abstract : Methanolic extract of stem bark of *Dichrostachys cinerea* was investigated for presence of bioactive constituents using column chromatography technique four major chemical constituents were isolated from the species and were analyzed using ^{1}H,^{13}C NMR ,FTIR and mass spectroscopy they were found to be 3,4-dihydro-2-(4-hydroxyphenyl-)-2H –chromene-3,4,8-triol, Fisetinidol,Mesquitol,Epicatechin ,were for the first time isolated from this species..

IndexTerms **- Mesquitol,Fisetinidol,Epicatechin,Bioactive constituents.**

I.INTRODUCTION:

Screening of natural sources such as plant extracts and fermentation products in search of new pharmacologically active compounds led to the discovery of many clinically usesful drugs that play key role in the treatment of human diseases. Bioactive principles were sought in earnest in the very early part of the nineteenth century,during which investigation of well known medicinal plants lead to the discovery of a number of biologically active alkaloids. Some of them like morphine, atropine, papaverine and codene became cornerstones of many aspects of drug discovery

II.LITERATURE REVIEW :

TABLE : List of compounds isolated from *Dichrostachys cinerea*

S.No	Compound name	Reference
1	Friedelin	1
2	Heptacosyl-p-coumarate	2
3	Friedelin-3 –β-ol	3
4	2-Methyl anthraquinone	4
5	3',4',5,7-Tetra hydroxyflavan	5
6	(-)-Liquiritigenin	6
7	Apigenin	7
8	(-)Naringenin	8
9	(-)-Fisetinidol	9
10	(-)-Mesquitol	10
11	(-)-Epicatechin	11
12	(-)-Naringenin5-O-B-D-glucopyranosid	12
13	5-O-[-LRhamnopyranosyl(12)--Larabinopyrano side	13
14	Betulinic acid	14
15	Hexacosanoic acid	15
16	5-Hydroxy-7, 3', 4'-trimethoxy5-O-α-L rhamnopyranosyl(1→2)-0-c-L-arabinopyranoside	16

PHARMACOLOGICAL REVIEW :

Treatment of *D. cinerea* root extract restores phosphate level, thus reducing the risk of stone formation. Administration of ethanolic and aqueous extract of *D.cinerea* root to urolithiatic rat, reduced and prevented the growth of urinary stone. This effect could conclude the anti-urolithiatic property and diuretic activity [17]of *D.cinerea Linn*.

Meroterpenes from root extract of *D.cinerea* inhibit Protein F amesyl Transferase Activity[18]. Protein farnesyl transferase (PFTase) plays an important role in the post-translational prenylation of several intracellular proteins .If the PFTase substrate is GTPase Ras, which is found in more than a third of human cancers in its mutated form, then the prenylation activity is inhibited, with functional consequences for the Ras-transformed cell phenotypes. As a result, PFTase inhibitors have been developed as potential anticancer drugs. The chloroform extract of the dried leaves of *Dichrostachys cinerea* was prepared and evaluated for the antibacterial and analgesic activities[19]. The tannins isolated from roots exhibited antibacterial activity against *Staph. aureus, Sh.boydii* and Sh. Flexneri, *E. coli* and *P. aeruginosa* . The inhibitory activities exhibited by the tannins tends to agree with the report that antibacterial properties of plants is due to the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils. The increase in antibacterial effectiveness observed with increase in concentration of tannins.

The ethanolic extract of the pods of *Dichrostachys cinerea* were found to posses anti oxidant activity[20].

III.OBJECTIVE:

From the literature review, it was observed that the plant was found to possess anti-urolithiatic, antibacterial and protein farnesyl transferase inhibitory activity. In the quest for the compounds responsible for the above mentioned activities the plant ***Dichrostachys*** *cinerea* was selected for the phytochemical investigation.

The objective of this research work was to carry out phytochemical investigation on the stem-bark of *Dichrostachys cinerea* in a scientific manner.

IV.MATERIALS AND METHODS:

COLLECTION OF PLANT MATERIAL:

The stem of *Dichrostachys cinerea* was collected from Sangam, near Hyderabad in the month of November 2017 and was identified by Dr. Radha Krishna, Department of Botany, Osmania University, Hyderabad.

EXTRACTION PROCESS :

The freshly collected plant stem was shade dried and dried stem (0.5 kg) was powdered and extracted with petroleum ether in a soxhlet apparatus for 30h and concentrated under reduce pressure in rotovap to obtain 8 g of the residue. And it was successively extracted with chloroform to yield 10 g of residue. After extracting with petroleum ether and chloroform the plant material was soaked in methanol at room temperature for 30h. The methanol was filtered and concentrated to obtain 5 g of residue.

PREPARATION OF COLUMN:

A cotton plug was placed at bottom of pre cleaned and dried column. Silica gel (60-120 mesh) was taken and mixed with chloroform to form a rapidly pourable mixture (slurry). It was poured in column and allowed to set.

PREPARATION OF SAMPLE:

The methanol extract (5 g) was dissolved in Acetone (100 ml) and silica gel was added (15 g). The acetone was removed under vacuum on rota vapour and the powder was transferred to a column of silica gel (60-120 mesh) set in chloroform and a cotton plug was placed on it.

GRADIENT ELUTION TECHNIQUE:

In order to isolate the compounds in a pure state from methanol fraction depending on its solubility, gradient elution technique was used. The column was eluted successively with chloroform first, chloroform: methanol (98: 2), chloroform: methanol (96: 4), chloroform : methanol (93 : 7) and chloroform : methanol (90 : 10). Fractions of 100 ml were collected. All the fractions were monitored by TLC. The TLC spots were visualized by various visualizing agents like 5%H_2SO_4,UV chamber, iodine chamber etc. The fractions were pooled according to their TLC patterns as follows:

TABLE : Fractionation table

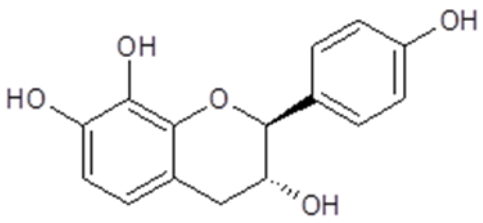
Eluent	Fraction No	Group No	Compound
Chloroform	1-10	**I**	-
Chloroform : methanol 98 :2	11-30	**II**	A
Chloroform: methanol 96 :4	31-45	**III**	B
Chloroform : methanol 93 :7	46-70	**IV**	C & D
Chloroform: methanol 90 :10	71-80	**V**	-

V.RESULTS AND DISCUSSION:

STRUCTURAL ELUCIDATION AND CHARACTERIZATION

STRUCTURE OF COMPOUND A:

Compound A was isolated as light brown solid, mp. 215-218°C, $[\alpha]_D$ -19.5°. Its molecular ion peak appeared at m/z 272.9 $(M-H)^+$and its molecular formula identified as $C_{15}H_{14}0_5$. The IR spectrum has shown a broad peak at 3466 cm^{-1} suggesting the presence of hydroxyl group indicated general nature of aromatic phenolic compound. The 300 MHz ^{1}H NMR Spectrum in methanol-d4 has shown two protons doublet at δ7.19 accounting for two aromatic protons (H-2' & 6'). And another two protons doublet at δ 6.76 accounting for two more aromatic protons (H-3' & 5').Two doublets at δ 6.39 (J = 8.0 Hz) and δ 6.37 (J = 8.0 Hz) each integrating for one proton are accounted for two more aromatic protons (H-5, 6). A one proton doublet at δ 4.72 (J = 7.5 Hz) and a proton multiplet at δ 4.04 are characteristic of oxymethine protons (H-2, 3). Two double doublets at δ 2.92 (J = 5.9 and 15.5 Hz) and 2.72 (J = 8.3 and 15.5 Hz) each integrating for one proton are characteristic of benenzylic methylene group (H-4). The large coupling constant of H-2 signal (J = 7.5 Hz) in the ^{1}H NMR spectrum indicates that the 4'-dihydroxy phenyl group at C-2 was in trans-position to the hydroxyl group at C-3. From the above data the compound was identified as 3,4-dihydro-2-(4-hydroxyphenyl)-2H-chromene-3,7,8-triol. Its ^{1}H and ^{13}C NMR data are summarized in table 4.



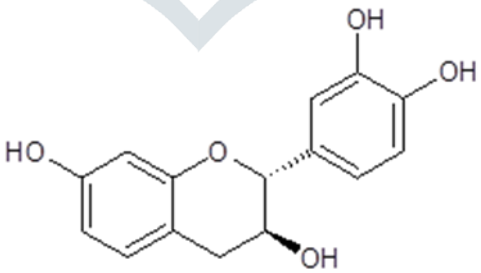
3,4-dihydro-2-(4-hydroxyphenyl)-2H-chromeme-3,7,8-triol

^{1}H and ^{13}C NMR spectral data of 3,4-dihydro-2-(4-hydroxyphenyl)-2H-chromene-3,7,8-triol

Position	^{1}H NMR(300 MHz, Methanol -d_4)δ(ppm)	^{13}c NMR 100 MHz Methanol-d_4	
		Position	δ (ppm)
H-2	4.72 (1H, d, J = 7.5 Hz)	2	82.4
H-3	4.04 (1H, m)	3	68.2
H-4eq	2.92 (1H, dd, J = 5-9 , 150 Hz)	4	32.8
H-4ax	2.72 (1H, dd, J= 8.3, 15.0 Hz)	4a	112.7
H-5	6.39 (1H, d, J= 8.5 Hz)	5	116.5
H-6	6.37 (1H, d, J= 8-5 HZ)	6	109.1
H-2'&6'	7.19 (2H, d, J = 8.5 Hz)	7	143.5
H-3&5'	6.76 (2H, d, J 8.5 Hz)	8	133.0
		8a	157.7
		1'	132.6
		2'	128.9
		3'	115.6
		4'	144.2
		5'	115.6
		6'	128.9

STRUCTURE OF COMPOUND B:

Compomd B was isolated as brown solid, m.p.208-210°C, $[\alpha]_D$ -9.5° and analyzed for $c_{15}H_{14}O_5$, (M^+ 273.2). The IR spectrum has shown a broad band at 3456 cm^{-1} suggesting the presence of hydroxyl group and indicated general nature of aromatic compound. The 300 MHz ^{1}H NMR spectrum in methanol-d_4 has shown two proton multiplet at δ 6.88 accounting for two aromatic protons (H-5, 2'). Two broad singlets at δ 8.30, 4.10 each integrating for a proton and a singlet at δ 8.01 integrating for two protons, which were exchanged with D_20 are characteristic of hydroxyl groups. Two double doublets at δ 6.74 (J =2.0, 8.0 Hz) and δ 6.38 (J = 2.0, 8.0 Hz) each integrating for one proton are accounted for two more aromatic protons (H-6', 6). Two doublets at δ 6.80 (J =8.0 Hz) and δ 6.30 (J= 2.2 Hz) each integrating for one proton are attributed respectively to H-5', 8. A one proton doublet at δ 4.62 (J = 7.6 Hz) and a proton multiplet at δ 4.05 are characteristic of oxymethine protons (H-2, 3). Two double doublets at δ 2.90 (J = 4.8 and 15.5 Hz) and 2.72 (J = 8.4 and 15.5 Hz) each integrating for one proton are characteristic of benzylic methylene group (H-4). The large coupling constant of H-2 signal (J = 7.6 Hz) in the ^{1}H NMR spectrum indicates that the 3', 4'-dihydroxy phenyl group at C-2 was in *trans*-position to the hydroxyl group at C-3.



(-)-Fisetinidol

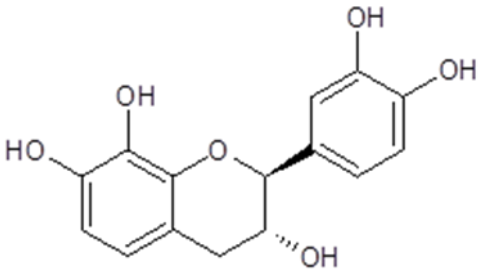
The ^{13}C NMR spectrum in methanol-d_4 has shown 15 carbons. Based on the above data along with its mass spectrum the structure of compound I was identified (-)-Fisetinidol[(-)-(2R, 3S)-2,3-trans-3',4',7-trihydroxy flavan-3-oI]. The data was compared with the reported values in literature[41] and it was found to be identical. It was previously reported from the same species

^{1}H and ^{13}C NMR spectral data of Fisetinidol

Position	^{1}H NMR(400 MHz, Methanol -d_4)δ(ppm)	^{13}c NMR 100 MHz Methanol-d_4	
		Position	δ (ppm)
H-2	4.62 (1H, d, J = 7.5 Hz)	2	83.1
H-3	4.01(1H, m)	3	69.0
H-4eq	2.89 (1H, dd, J = 5.0 , 15.0 Hz)	4	33.7
H-4ax	2.72 (1H, dd, J= 8.0, 15.0 Hz)	4a	112.6
H-5	6.40 (2H,S)	5	131.5
H-6	6.40 (2H,S)	6	110.0
H-2’	6.88 (1H, d, J = 2.0 Hz)	7	157.7
H-5’	6.80 (1H, d, J =2.0,8.0 Hz)	8	104.1
H-6’		8a	156.1
		1’	131.8
		2’	115.4
		3’	146.2
		4’	146.0
		5’	116.4
		6’	120.4

STRUCTURE OF COMPOUND C:

Compound C was obtained as pale brown semi solid, m.p. 252-254°C, $[\alpha]_D$ 36.05° and analyzed for $C_{15}H_{14}0_6$ (M^+ 290) which was further confirmed by its El-MS. The IR spectrum has shown a sharp band at 3448 cm^{-1} indicating the presence of hydroxyl group and suggested the general nature of an aromatic compound. The 300 MHz ^{1}H NMR spectrum in acetone-d_6 has shown two doublets at δ 6.88 (J 2.0 Hz), 6.80 (J = 8 Hz) and a double doublet at δ.74 (J = 2, 8.0 Hz) each integrating for one proton indicated the presence of a 1,2,4-trisubstituted aromatic ring (H-2’,5',6'). A singlet at δ 6.40 integrating for two protons suggested the presence of two aromatic protons (H-5, 6). Three singlets at δ 7.95 (2H), 7.55 (1H) and 7.25 (1H), which were exchanged with D_20 are characteristic of phenolic hydroxyl groups. A one proton doublet at δ 4.62 (J = 7.2 Hz) was attributed to oxymethine proton (H-2). A broad singlet integrating for two protons at δ 4.01, which upon D_20 exchange observed as one photon multiplet accounting for the presence of hydroxy methine proton (H-3) as well as hydroxyl group (OH-3). Two double doublets at δ 2.89 (J= 5.0 and 15.0 Hz) and δ 2.71 (J= 8.0 and 15.0 Hz) each integrating for one proton, indicated the presence of benzylic methylene (H-4)



(-)-Mesquitol

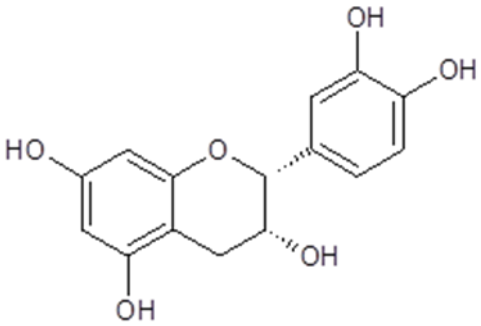
The ^{13}C NMR spectrum in acetone-d_6 has shown 15 carbons. Based on the above data along with its mass Spectrum. The compound C was identified as (-)-mesquitol[42] , which is an isomer of (+)-mesquitol [(2R, 3S)-2,3-trans-3',4',7,8-tetrahydroxy flavan-3-ol][43]. The 3',4'-dihydroxy phenyl group at C-2 was in trans-position to the hydroxyl group at C-3 according to the large coupling constant of H-2 signal (J = 7.2 Hz) in the ^1H NMR spectrum of (-)-mesquitol, which was coincident with that of a (+)-mesquitol moiety (J = 7.2 Hz). Hence the configuration at C-2 and C-3 is established as 2S, 3R respectively. The optical rotation of (-)-mesquitol has shown equal and opposite sign to that of (+)-mesquitol. Hence the (-)-mesquitol [(2S, 3R)-2,3-trans-3',4',7,8-tetrahydroxy flavan-3-ol] is presumed to be the other enantiomer of (+)-mesquitol.

^1H and ^{13}C NMR spectral data of Mesquitol

Position	^1H NMR(300 MHz, Methanol –d_6) δ (ppm)	^{13}c NMR 100 MHz Methanol-d_6	
		Position	δ (ppm)
H-2	4.62 (1H, d, J = 7.5 Hz)	2	80.9
H-4eq	2.89 (1H, dd, J = 5.0 , 15.0 Hz)	3	68.1
H-4ax	2.72 (1H, dd, J= 8.0, 15.0 Hz)	4	34.6
H-5	6.40 (2H,S)	4a	113.3
H-6	6.40 (2H,S)	5	114.2
H-2’	6.88 (1H, d, J = 2.0 Hz)	6	109.9
H-5’	6.80 (1H, d, J =2.0,8.0 Hz)	7	144.5
H-6’	6.74(1H,dd,J=2.0 and 8.0 Hz)	8	134.3
		8a	145.7
		1’	132.8
		2’	118.6
		3’	146.5
		4’	146.7
		5’	116.1
		6’	116.6

COMPOUND D:

Compound D was obtained as amorphous powder, m.p. 242-244°C, [αl_D-68.4° and analyzed for $C_{15}H_{14}0_6$ [$(M+H)^+$ 291]. The IR spectrum has shown a broad band at 3443 cm^{-1} indicating the presence of hydroxyl group and suggested the general nature of aromatic compound. The 300 MHz ^1H NMR spectrum in methanol-d_4 has shown a one proton doublet at δ7.02 (J = 1.8 Hz) and a multiplet at δ 6.81 integrating for two protons are characteristic of aromatic protons (H-2', 5', 6'). Three singlets at δ 8.26 (1H), 8.08 (1H) and 7.90 (2H) which were exchanged with D_20 are characteristic of phenolic hydroxyl groups. Two doublets at δ 6.01 (J = 2.0 Hz) and δ 5.92 (J = 2.0 Hz) each integrating for one proton suggested the presence of two more aromatic protons (H-8, H-6). A one proton doublet at δ 4.86 (J = 1.0 Hz) and a multiplet at δ 4.20 (1H) were indicative of two oxymethine protons (H-2, H-3). A doublet integrating for a proton at δ 3.61 (J = 5.2 Hz) which was exchanged with D_20 was accounted for hydroxyl group (3-OH). Two double doublets at δ 2.85 (J = 4.5 and 16.0 Hz) and 2.74 (J = 4.5, 16.0 Hz) each integrating for a proton are attributed to benzylic protons (H-4). The minor coupling constant of H-2 signal (J = 1.0 Hz) in the ^1H NMR spectrum indicates the 3', 4'-dihydroxyphenyl group at C-2 was in cis-position to the hydroxyl group at C-3.



(-)-Epicatechin

The ^{13}C NMR in acetone-d_6 has displayed 15 carbons. Based on the above data along with its mass spectrum the structure of compound D was identified as (-)-epicatechin [(2R, 3R)2,3 cis 3', 4',7,8 tetrahydroxy flavan-3-ol] . The data was compared with reported values in literature [44] and it was found to be identical. The occurrence of (-)-epicatechin is first time reported from this genus

^{1}H and ^{13}C NMR spectral data of Epicatechin

Position	^{1}H NMR(300 MHz, Methanol –d_6) δ (ppm)	^{13}c NMR 100 MHz Methanol-d_6	
		Position	δ (ppm)
H-2	4.86(1H, d, J = 1.0Hz)	2	76.2
H-3	4.20(1H,m)	3	67.7
H-4eq	2.85(1H, dd, J = 4.5 , 16.0 Hz)	4	27.7
H-4ax	2.74 (1H, dd, J= 4.0, 16.0 Hz)	4a	99.6
H-5	6.40 (2H,S)	5	156.0
H-6	5.92 (1H, d, J = 2.0 Hz)	6	95.3
H-8	6.01(1H, d, J =2.0 Hz)	7	156.5
H-2’	7.02(1H,d,J=1.8 Hz)	8	94.6
H-5’	6.81(2H,m)	8a	155.7
H-6’	6.81(2H,m)	1’	130.8
		2’	114.0
		3’	144.6
		4’	114.5
		5’	114.7
		6’	119.2

CONCLUSION:

Detailed description and structural elucidation , characterization of isolated compounds 3,4-dihydro-2-(4-hydroxyphenyl)-2H-chromene-3,7,8-triol, Fistinidol, Mesquitol and Epicatechin from *DICHROSTACHYS CINEREA* was made the compound 3,4-dihydro-2-(4-hydroxyphenyl)-2H-chromene-3,7,8-triol was isolated for the first time from this species.

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