Biological and Chemical Studies on *Calycopteris floribunda* leaves

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ABSTRACT: Dichloromethane-methanol extract of leaves of *Calycopteris floribunda* and its aqueous 90% methanol soluble fractions showed significant antibacterial activity against *Bacillus subtilis, Streptococcus pyogen, Staphyloccus aureus* and *Salmonella typhi*. The aqueous 90% methanol and 1-butanol soluble fractions of the leaves showed significant β-glucuronidase inhibition and antioxidant activity. Two pure compounds, 3,8-di-*O*-methyl ellagic and 2,3,7-tri-*O*-methyl ellagic acids were isolated from the 1-butanol soluble fraction of the parent extract.

Key words: *Calycopteris floribunda,* Combertaceae, antibacterial, antioxidant, β-glucuronidase, ellagic acid.

INTRODUCTION

Calycopteris floribunda Lamk (Combertaceae) is an evergreen shrub, locally known as guicha lata. The plant is being used medicinally for various complications.[1-3] A number of phenolic and non phenolic flavonoids including cytotoxic, anthelmintic and antiviral properties have been isolated from the plant.[4-5] Earlier we reported cytotoxicity and isolation of steroid and terpene from the leaves of the plant.[6] In continuation of our work on *Calycopteris floribunda*, we are now reporting antibacterial, β-glucuronidase inhibition and antioxidant activity of the plant along with isolation of ellagic acids and its analog.

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MATERIALS AND METHODS

General Methods. All evaporations were carried at bath temperature not exceeding 40° C. Melting point was determined with a MEL-TEMP II apparatus (USA). IR spectra were recorded on a Shimadzu IR-470 spectrometer. ^{1}H & ^{13}C NMR spectra were recorded using a 400 MHz Bruker NMR Spectrometer with TMS as the internal standard. Mass spectra were recorded on a Esquire mass spectrometer. Silica gel (60G, 70-230 mesh, particle size 0.043-0.063 mm) was used for column chromatography. TLC was carried out on precoated silica gel (PF-254) on aluminum sheets.

Plant Materials. Fresh plants (*C. floribunda*) were collected from Sarkarhat of Hatazari thana of Chittagong district. The plant was identified by late Professor M. Salar Khan of Bangladesh National Herbarium (BNH). A voucher specimen of the plant was deposited at BNH (BNH Accession No. 27997). The collected plants were cleaned, air dried and finally dried at oven at 40^{0} C. The dried leaves were ground to a powder with a cyclotec grinder.

Extraction. The powdered leaves (4 kg) of *Calycopteris floribunda* was extracted with CH_2Cl_2-CH_3OH (1:1, 3 X 8L, 24 h. RT). The extract was filtered, concentrated and evaporated to dryness, yielding 146 g extract. The extract was suspended in H_2O and partitioned with CH_2Cl_2 (1L X 3), yielding CH_2Cl_2 extract (80 g). The CH_2Cl_2 extract was again suspended in aqueous 90% MeOH and partitioned with n-hexane (1L X 3), yielding hexane (36 g) and aqueous 90% MeOH (42 g) extract. The aqueous part (56 g) was also partitioned with 1-butanol to get 1-BuOH soluble part (25 g).

Isolation. 1-Butanol soluble fraction (25 g) was fractionated into 6 fractions (Bu-1-1 ↔Bu-1-6) by Sephadex LH-20 column using H_2O and MeOH as eluent. The sub fraction Bu-1-4 (2.5 g) was again chromatographed on reversed phase RP-18 column using mobile phase H_2O and MeOH to get six fractions (Bu-2-1-↔Bu-2-6). The fraction Bu-2-4 (500 mg) was further fractionated into 6 fractions (Bu-3-1↔-Bu-3-6) by silica gel column chromatography using hexane, DCM and MeOH as eluent. The fractions Bu-3-2 and Bu-3-3 gave compound 1 and compound 2 by repeated wash with hexane.

3,8-Di-*O*-methyl ellagic acid (1): Pale yellow solid, R_f value : 0.53 MeOH in CH_2Cl_2 (1:8), m.p: 312-315° C.

^{1}H NMR (DMSO d_6):10.5 (2H, bs, OH-2, OH-7), 7.5 (1H, s, H-6), 7.3 (1H, s, H-1), 4.04 (6H, s, OCH_3-3, OCH_3 -8).

^{13}C NMR (DMSO d_6): 107.3 (C-1), 148.1 (C-2), 140 (C-3), 136 (C-3a), 158 (C-5), 111 (C-5a), 111.7 (C-6), 152 (C-7), 141.3 (C-8), 139.6 (C-8a), 158.7 (C-10), 112 (C-10a), 111.7 (C-11), 112.3 (C-12), 60.9 (OMe-3), 60.9 (OMe-8).

2,3,7-Tri-***O*-**methyl ellagic acid (2): Pale yellow solid, R_f value : 0.48 MeOH in CH_2Cl_2 (1:6), m.p: 293-295° C.

^{1}H NMR (DMSO-d_6) : 8.3 (1H, s, OH), 7.6 (1H, s, H-1), 7.5 (1H, s, H-8), 4.05 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.0 (3H, s, OCH_3).

^{13}C NMR (DMSO-d_6) : 107.5 (C-1), 140.8 (C-2), 141.0 (C-3), 141.4 (C-3a), 158.3 (C-5), 113.4 (C-5a), 153.8 (C-6), 140.2 (C-7), 111.7 (C-8), 141.0 (C-8a), 158.5 (C-10), 112.5 (10a), 111.0 (C-11), 112.7 (C-12), 56.7 (OMe-2), 61.3 (OMe-3) and 61.5 (OMe-7).

EIMS : 344 (M^+) , 327, 306 , 286, 172 , 103, and 74

BIOLOGICAL SCREENING

Antibacterial Assay. The DCM-MeOH (1:1) extract and its aqueous 90% MeOH & 1-BuOH soluble fractions were studied for antibacterial activity against six different bacterial strain, *Bacillus subtilis*, *Streptococcus pyogen, Staphyloccus aureus* (Gram-positive bacteria) and *Escherichia coli Pseudomonas aerogenes* and *Salmonella typhi* (Gram-negative bacteria) following agar diffusion protocol[7] (Table 1).

Table 1. Antibacterial activity of the extract of *C. floribunda* leaves and its fractions

Name of Bacteria	Name of extract	Zone inhibition (mm)	Refn. Drug.
Bacillus subtilis,	CH_2Cl_2:MeOH	21	
	Aqueous 90% MeOH extract	18	34
	1-Butanol	15	
Streptococcus pyogen	CH_2Cl_2:MeOH	19	
	Aqueous 90% MeOH extract	18	30
	1-Butanol	16	
Staphyloccus aureus	CH_2Cl_2:MeOH	19	
	Aqueous 90% MeOH extract	23	30
	1-Butanol	16	
Escherichia coli	CH_2Cl_2:MeOH	14	
	Aqueous 90% MeOH extract	16	30
	1-Butanol	16	
Pseudomonas aerogenes	CH_2Cl_2:MeOH	14	
	Aqueous 90% MeOH extract	16	28
	1-Butanol	15	
Salmonella typhi	CH_2Cl_2:MeOH	19	
	Aqueous 90% MeOH extract	23	30
	1-Butanol	14	

Activity key: 11-14 mm inactive; 15-17 mm good activity, 18-above, significantly active.

β-Glucuronidase Inhibition Activity. ß-Glucuronidase inhibition activity of the parent extract and its aqueous 90% MeOH, 1-butanol soluble

fractions were done according to standard method[8] (Table 2).

Antioxidant activity. Antioxidant activity of the DCM-MeOH (1:1) extract, aqueous 90% MeOH and 1-butanol soluble fractions of the leave and stem were studied with DPPH free radical scavenging activity according to standard method.[9] (Table 3).

Table 2. β-Glucuronidase Inhibition activity of the extract of *C. floribunda* leaves and its fractions

Name of plant	Name of extract	% Inhibition in extract	% Inhibition in standard drug
Calycopteris	DCM-MeOH	17	
floribunda	Aq 90% MeOH	64	100
	1-Butanol	80	

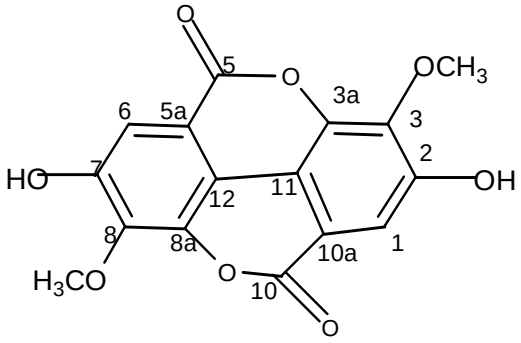
Table 3. Antioxidant activity activity of the extract of *C. floribunda* leaves and its fractions

Name of plant	Plants part	Name of extract	% Scavenging activity
Calycopteris		DCM-MeOH	66
floribunda	Leaf	Aq 90% MeOH	89
		1-Butanol	92

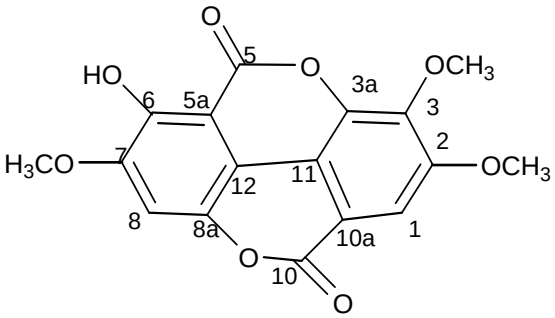
DPPH free radical scavenging activity: Criteria: 0-30 %, inactive; 30-50 % little active; 50-70 % moderate; above 70% highly active

RESULTS AND DISCUSSION

Compound **1** was isolated as a pale yellow amorphous solid from butanol fraction by column chromatography. ^{1}H NMR of the compound **1** revealed a signal at δ_H 10.5 (2H,bs) for two aromatic hydroxyl protons. Two aromatic protons appeared at δ_H 7.39 (1H, s) and 7.34 (1H, s) and one signal at δ_H 4.04 (6H, s). ^{13}C spectrum of the compound I had 15 signals in which the most up field signal at δc 60.92 is for –OCH_3 group. The signal is doubly intense than the other signals indicate that two OCH_3 group in the compound. Two downfield signals at δc 158.67 and 158.77 ppm were assigned to two carbonyl carbons at C-5 and C-10 positions, respectively. The signals at δc 148.9 and 151.99 ppm were due to carbon attach with hydroxyl group at C-2 and C-7. The other two signals at δc 140.06 and 141.30 ppm were due to the attachment of –OCH_3 group at C-3 and C-8. ^{1}H and ^{13}C NMR data of the compound **1** was compared with reported compound[10,11] and the compound was identified as 3,8–di-*O* –methyl-2,7-dihydroxy ellagic acid.



Compound 1 (3,8-Di-*O*-methyl ellagic acid)



Compound 2 (2,3,7-Tri –*O*-methyl ellagic acid)

Compound **2** was isolated as a pale yellow solid from the 1-butanol fraction by column chromatography. It had melting point 293-295^{0}C and soluble in dimethyl sulphoxide (DMSO). ^{1}H NMR of the compound **2** revealed a signal at δ_H 8.30 (1H, s) for aromatic hydroxyl proton. Two aromatic protons appeared at δ_H 7.60 (1H, s) and 7.52 (1H, s) and three signals at δ_H 4.05 (3H, s, OCH_3), 4.04 (3H, s, OCH_3) and 3.99 (3H, s, OCH_3) for three -OCH_3 groups present in the compound. The EIMS of the compound 2 showed that the molecular ion peak at *m/z* 344. ^{13}C spectrum of the compound had 17 carbon signals indicating that the compound contained 17 carbons. The molecular formula of the compound was ascertained as $C_{17}H_{12}O_8$. The above fact indicated that the compound might be a derivative of ellagic acid. Two downfield signals at δc 158.29 and 158.47 ppm were assigned to two carbonyl carbons at C-5 and C-10 positions. The signal at 153.76 ppm was due to carbon, which contain a hydroxyl group at C-6

position. The two -CH_2 signals from DEPT experiment at 107.45 and 111.68 was due to two methylene groups at C-1 and C-8 position in the compound. The most up field signals at 56.73, 61.31 and 61.47 were due to three –OCH_3 groups attached to C-2, C-3 and C-7 carbon atoms. Compound **2** was compared with the reported compounds[10,11] and was identified as 2,3,7-tri-*O*-methyl ellagic acid. Both the compounds were isolated for the first time from this plant.

The leaves extract was found to possess cytotoxic to brine shrimp lethality assay as well as by cell line screening.[6] The same extract and its aqueous 90% methanol soluble fractions showed significant antibacterial activity against *Bacillus subtilis, Streptococcus pyogen, Staphyloccus aureus* and *Salmonella typhi* . But 1-butanol soluble fraction of the extract was found to be moderately active.

The 1-butanol soluble fractions of the leaves showed significant β-glucuronidase inhibition activity whereas the parent extract was found to be inactive and the aqueous 90% methanol soluble fractions was less active than the 1-butanol soluble part. The result indicated that the more polar compounds which are present in the 1-butanol extract might be responsible for β-glucuronidase inhibition effect.

Similar effect was also found in antibacterial assay where the 1-butanol soluble fraction showed highest antioxidant activity than the parent extract and its aqueous 90% methanol soluble fraction.

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