Indian Journal of Chemistry
Vol. 61, September 2022, pp. 989-993
DOI: 10.56042/ijc.v61i9.66352

A new cinnamic ester derivative from *Glochidion velutinum* leaves and evaluation of its antioxidant and antibacterial activity

Ananta Kumar Das[a,*], Jesmin Khan[a], Mesbah U Ahmad[a], Ataur Rahman[b] & Md Emdad Hossain[c]

[a] Department of Pharmacy, Gono Bishwabidyalay, Savar, Dhaka-1344

[b] Department of Chemistry, Jahangirnagar University, Savar, Dhaka-1343

[c] Wazed Miah Science Research Centre, Jahangirnagar University, Savar-1343

*E-mail: anantaraj1473@gmail.com

Received 1 December 2020; accepted (revised) 1 August 2022

The plant *Glochidion velutinum* (GV) family *Euphorbiaceae*, a medicinal plant, is reported to possess various therapeutic applications such as antidiabetic, anticancer, antioxidant, antimicrobial, anti-inflammatory, *etc*. However, the compounds responsible for therapeutic value have not yet been isolated. The present study reports the isolation of a compound from the leaves of GV. The compound is identified as a cinnamic acid derivative (*p*-pentadecayl ethyl cinnamate) through extensive spectral studies. Its antioxidant nature such as DPPH free radical scavenging assay, total antioxidant capacity, and antibacterial activities have been evaluated. The compound exhibited lower antioxidant properties compared to the standard. It has shown moderate antimicrobial activity against ten bacteria and significant antibacterial activity against *Proteus vulgaris* compared to standard ciprofloxacin.

Keywords: *Glochidion velutinum*, cinnamic acid, antioxidant, DPPH, antimicrobial, *Proteus Vulgaris*

Plants produce many chemicals that are biologically active not just in themselves but also in other organisms. Herbs and plants with medicinal properties play a vital role in the remedies of various ailments[1]. Plants have been used for medicinal purposes long before the prehistoric period. The Indian subcontinent is known to be a vast resource of medicinal plants[2]. *Glochidion* (Family-Euphorbiaceae) is a very vast genus of more than 250 species including *Glochidion velutinum* (GV). Most of the species under this genus are widely constituted with flavonoids and triterpenoid saponins[3]. This group of plants is well known among the tribes for its anti-cancer, hypotensive, diuretic, and various other properties[3]. GV is a small tree or a large shrub up to 9 m with coraceous, pinnate venation leaves, yellow male flowers, and globose and depressed fruit branches and leaves. This plant is widely distributed in China, Pakistan, India, Nepal, and Bangladesh[4-6].

Several triterpenoids, triterpenoid glycosides, and alkaloids are known to be the constituents of this plant[3]. The stem bark of the plant is identified as a moderate bactericidal[7]. The traditional claim is that this plant is used as cancer, diabetes, anti-diarrheal agent and against inflammation and for the healing of wounds[9]. Some work reported that the leaves of GV are effective as antidiabetic, anticancer, antioxidant, antimicrobial, anti-inflammatory, cytotoxic, *etc*.[3,8-13]. A previous study of this laboratory on this plant showed that the methanol extract of GV possesses significantly better activity than the standard ones as well as compared to the other extracts *e.g*. hexane, chloroform. It has a higher phenolic and flavonoid contents[7]. However, no pure compound from the plant extracts has yet been isolated. The present study was directed towards the isolation of bioactive constituents of the leaves and to evaluate their pharmacological property.

Results and Discussion

The tannin-free methanol extract of GV leaves was partitioned successively with diethyl ether, ethyl acetate, and butanol. While diethyl ether and butanol extracts were showed to be complex mixtures, the ethyl acetate extract was rather less complex. The ethyl acetate extract was subjected to preparative TLC to yield a pure cinnamic acid ester derivative compound-1 (Fig. 1).

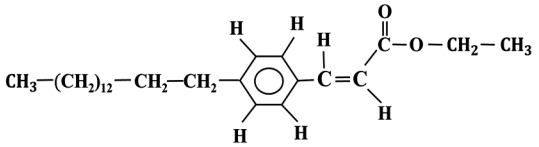

Fig. 1 — Compound-1 (*p*-pentadecayl ethyl cinnamate)

Table 1 — Antioxidant activity of compound 1 and ascorbic acid

Conc. (µg/mL)	Absorbance of ascorbic acid	% Inhibition of ascorbic acid	IC_{50} value of ascorbic acid (µg/mL)	Conc. (µg/mL)	Absorbance of compound 1	% Inhibition of compound 1	IC_{50} value of compound 1 (µg/mL)
50	0.031	97	4.64	400	0.427	57	302.5
25	0.058	94		200	0.561	44	
12.5	0.241	76		100	0.649	35	
6.25	0.451	55		50	0.697	30	
3.125	0.579	42		25	0.723	28	
1.56	0.719	38		12.5	0.748	25	
-	-	-		6.25	0.772	23	

*Absorbance of 0.004% DPPH (control) = 1.004

The compound was assigned to the molecular formula $C_{26}H_{42}O_2$ (based on ^1H and ^{13}C NMR). UV-visible spectrum of the compound exhibited an absorption maximum of 310 nm which indicated the characteristic absorption for the presence of a conjugated system. The compound was an ester is indicated by a strong absorption at 1706.07 cm^{-1} in its IR spectrum. The presence of an aromatic nucleus was obvious from the IR absorption bands between 1515.11- 1605.77 cm^{-1} for the skeletal vibration. Besides these, absorptions due to C-H were clearly shown by bands at 2918.35 and 2849.87 cm^{-1}. The ^1H NMR spectrum of the compound showed a 2H quartet at δ 4.30 and a 3H triplet at δ 1.37 which indicated the presence of a methylene group and methyl group of an ethyl ester function. A 2H triplet at δ 2.39 and a 3H triplet at δ 0.92 are appropriate for benzylic methylene protons attached to a CH_2 group and a terminal methyl group of an aliphatic chain, respectively. The 24H huge unsplit singlet at δ 1.28 reveals the presence of a long aliphatic chain in the molecule. A 2H multiplet at δ 1.68 indicated the presence of a methylene group which is bonded with the benzylic carbon atoms and the long aliphatic chain, respectively. Two 2H doublets at δ 6.87 (J= 8.4 Hz) and δ 7.46 (J=8.8 Hz) revealed the presence of a *p*-disubstituted benzene ring in the molecule. Another two doublets at δ 6.34 (J=16.0 Hz) and δ 7.67 (J=15.6 Hz) clearly show the presence of two olefinic protons in the molecule which are *trans* to each other. The ^{13}C NMR signals at 167.42, (114.94, 115.80, 129.92, 129.96, 132.35, 143.14) are consistent with the carbonyl C of the ester function and six aromatic carbon atoms respectively. Whereas the signals at 115.86 and 144.24 are appropriate for olefinic C and the signal at 60.39 for O-CH_2 carbon atom. The other C signals at 14.10, 14.35, 22.00, 24.73, 29.075, 29.24, 29.36, 29.44, 29.59, 29.639, 29.67, 29.68, 2×29.70, 31.93, and 33.59 are attributable to aliphatic C atoms.

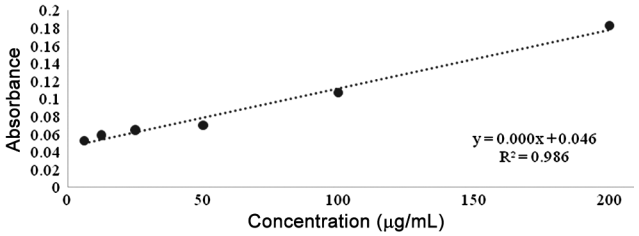

Fig. 2 — Calibration curve of ascorbic acid

The suggested structure is confirmed by the ^1H-^1H cosy spectrum. ^1H -^1H cosy spectrum of the compound is consistent with 1H olefinic proton that give a doublet at δ 7.67 coupled to 1H olefinic hydrogen atom also as a doublet at δ 6.34. The two aromatic hydrogen atoms that give a doublet at δ 7.46 and at δ 6.87 are coupled to *o*-H atom. The two aliphatic methylene hydrogen atoms bonded to the oxygen atom of an ester function whose signal appears as a quartet at δ 4.30 are coupled to the methyl protons that gives a triplet signal at δ 1.37. The 2H triplet signal at δ 2.39 is connected to the 2H methylene protons that give signal as a multiplet at δ 1.68. The three methyl protons that give a signal as a triplet at δ 0.92 are connected to the methylene singlet that appears at δ 1.28.

Antioxidant activity of the compound (*p*-pentadecyl ethyl cinnamate)

The total antioxidant capacity of the compound is 15.372 mg/g. It showed a less Mo (VI) reducing ability that indicates the compound showed lower antioxidant activity. IC_{50} value of DPPH free radical scavenging assay of compound is 302.5 µg/mL whereas that of ascorbic acid is 4.64 µg/mL which indicates rather mild antioxidant activity of the compound (Table 1 & Figs 2 & 3).

Antibacterial activity of the compound 1

The antibacterial activity of the compound was assayed against 14 bacteria. Results showed that *Bacillus subtilis, Klebsiella pneumoniae, Salmonella*

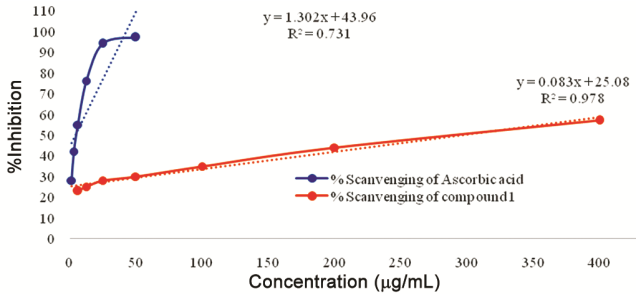

Fig. 3 — DPPH free radical scavenging assay of compound 1 and ascorbic acid

Table 2 — Antibacterial activity of compound 1 and Ciprofloxacin

Test Bacteria	Ciprofloxacin	Compound 1	NC
Bacillus Subtilis	27	-	-
Bacillus Cereus	25	12	-
Staphylococcus Aureus	22	9	-
Enterococcus Faecalis	35	10	-
E.coli EPEC	32	8	-
E.coli ETEC	27	5	-
E. coli ATCC	33	8	-
Klebsiella Pneumoniae	25	-	-
Klebsiella Oxytoca	25	8	-
Klebsiella Michiganensis	18	7	-
Klebsiella SPP	34	11	-
SalmonellaTyphi	30	11	-
SalmonellaParatyphi	28	-	-
Proteus Vulgaris	10	10	-

*** (-) indicate no zone of inhibition

paratyphi did not respond to the compound. Other organisms show mild to moderate activity compared to standard. The compound showed significant activity against *Proteus vulgaris* compared to standard ciprofloxacin (Table 2).

Experimental Section

Plant Material

The leaves of the plant were collected from the forest range of Rajendrapur, Gazipur, Dhaka, Bangladesh during the 2nd week of January 2015 and were identified at the Bangladesh National Herbarium, Mirpur, Dhaka-1205. The accession number is DACB-43211. The leaves were cleaned to remove dust and other undesirable materials. The cleaned leaves were dried in shade for 7 days followed by drying in an oven at 40 °C for 2 h. The leaves were then reduced to a coarse powder using a grinder.

Chemicals and instrumentations

The solvents used were of analytical/ reagent grade (Merck). DPPH (2,2´-diphenyl -1-picrylhydrazyl) was purchased from Sigma Aldrich. All other reagents were analytical grade and purchased from Merck. Precoated silica gel 60 F_{254} plates (Merck) 0.2 mm were used for TLC analysis as well as PTLC. The IR spectra were recorded on Shimadzu Model no. IRtracer-100 AH FTIR spectrometer as KBr pellets. UV-visible spectra were recorded on a Shimadzu UV1800 series, UV-visible double beam spectrophotometer in MeOH. NMR spectra were recorded on a Bruker 400 spectrophotometer operating at 400 MHz for ^{1}H NMR and ^{1}H-^{1}H cosy and 100 MHz for ^{13}C NMR using $CDCl_3$ as solvent, TMS as internal standard of the NMR at Wazed Miah Science Research Centre, Jahangirnagar University, Savar, Dhaka-1343.

Extraction and isolation

400 g of powdered dried leaves of *Glochidion velutinum* were extracted successively with n-hexane, chloroform, methanol, and ethanol (95%) by hot extraction method in a Soxhlet. The crude yields of these extractions amounted to 7.18 g (GVH), 7.22 g (GVC), 95 g (GVM), 22 g (GVE) respectively on removal of solvents at 40 °C. The present work was concentrated on the separation of the individual components of methanol extract (GVM) which in an earlier study exhibited pronounced pharmacological effect compared to standard[7].

Study on methanol extract (GVM)

The methanol extract, a reddish-black solid, was sparingly soluble in solvents like dichloromethane, ethyl acetate, and chloroform and completely soluble in methanol and ethanol. The TLC examination in different developing solvents gave a poor resolution, a long tail from the baseline. The extract showed a positive tannin test with lead acetate.

Removal of tannin from GVM

10 g of crude methanol extract was dissolved in 150 mL of ethanol. Part of the extract did not go into the solution. The undissolved part (1.24 g) was removed by filtration. The clear ethanol-soluble part was then treated with 5% sodium hydroxide (30 mL) when tannin precipitated out. The precipitated tannin was removed after filtration[13]. The clear solution was further centrifugated to remove any undissolved material. The tannin precipitate was dried, brownish-black in colour amounting to 5.58 g. The tannin-free supernatant of the centrifugate was then diluted with 150 mL distilled water. The diluted aqueous ethanolic

solution was extracted successively with diethyl ether, ethyl acetate, and butanol. The diethyl ether and ethyl acetate fractions were dried over anhydrous sodium sulfate to remove adhering water. Sodium sulfate was removed by filtration and solvent was removed on a rotary evaporator at 40 °C. TLC examinations showed that the diethyl ether and butanol extracts were complex mixtures, whereas the ethyl acetate extract was less complex showed two major spots.

Study on ethyl acetate fractions

The ethyl acetate extract was a deep brown, solid, 87 mg. It was soluble in ethyl acetate, dichloromethane, and methanol. TLC examination showed the best resolution in (n-hexane: ethyl acetate 3:2) for the presence of two components with R_f 0.50, 0.72.

Separation of component of ethyl acetate (EtOAc) fraction by preparative thin layer chromatography

The EtOAc extract was dissolved in a minimum quantity of ethyl acetate and subjected to preparative thin-layer chromatography (PTLC) on silica plates using ethyl acetate and n-hexane (2:3) as the developing solvent. After completion of development, the silica gel adsorbent corresponding to the zones R_f 0.72, 0.5 were carefully removed from the plate. The adsorbents corresponding to the two zones were extracted with ethyl acetate. The solvent was removed from the extracts on a rotary evaporator. The process yielded 45mg of colourless viscous oil (A) with a R_f value of 0.72 and 5mg (B) with R_f 0.5 as colourless solid in n-hexane: ethyl acetate (3:2).

Further TLC studies of A showed that it contained more than two compounds. Fraction B was white solid (5 mg). It exhibited a single spot on silica gel TLC plates in different solvent systems, *e.g.* gradient mixture of n-hexane and ethyl acetate, as well as dichloromethane. In all the solvent systems it showed one spot suggesting it to be a pure compound.

Study of the compound

UV-visible spectrum showed maximum absorption at 310 nm; IR (KBr) ν_{max} 2918.35, 2849.87, 1369.48, 1450, 1515.11, 1605.77, 1706.07 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ (ppm) δ 4.30 (2H, quartet), δ 1.37 (3H, δ triplet), δ 2.39 (2H triplet), δ 0.92 (3H triplet), δ 1.28 (24H, singlet), δ 1.68 (2H multiplet) δ 6.87 (2H, J=8.4), δ 7.46 (2H, J=8.8), δ 6.34 (2H doublets, J=16.0 Hz), δ 7.67 (2H doublet, J=15.6). ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) spectrum of compound showed 26 signals. The ^{13}C NMR signals are as follows: 14.10, 14.35, 22.00, 24.73, 29.075, 29.24, 29.36, 29.44, 29.59, 29.639, 29.67, 29.68, 2×29.70, 31.93, 33.59, 60.39 (0-CH_2), 115.86 (olefinic), 144.24 (olefinic), 114.94, 115.80, 129.92, 129.96, 132.35, 143.14, 167.42(C=0).

Antioxidant study - total antioxidant capacity

The phosphomolybdenum method was employed to determine the total antioxidant capacity. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acid *p*H with a maximal absorption at 695 nm[14].

$$Mo\ (VI) + e \rightarrow Mo\ (V)$$

The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following formula equation

$$A = (c \times V)/m,$$

Where, A = total content of Antioxidant compounds mg/g of compound 1, in Ascorbic acid Equivalent, c = the concentration of Ascorbic acid established from the calibration curve, mg/mL, V = the volume of compound in mL, m = the weight of the isolated compound, g.

Antioxidant study - DPPH free radical scavenging activity

DPPH is a stable free radical that acts as an electron acceptor (oxidizing agent) and causes oxidation of other substances. On the other hand, antioxidants act as electron donors (reducing agent). Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as dark-coloured crystalline powder and forms deep violet colour in solution. The scavenging of DPPH free radical is indicated by the deep violet colour being turned pale yellow or colorless[15]. Various concentrations (6.25 to 400 µg/mL) of the compound were used to assess free radical inhibitory ability and ascorbic acid (1.56 to 50 µg/mL) was taken as standard. The absorbance of compound/standard was measured at 517 nm with UV-visible spectrophotometer (Shimadzu) and their DPPH free radical inhibitory:

$$\%\ \text{inhibition} = (C-T)/C \times 100$$

Where, C=Absorbance of control and T= Absorbance of compound/standard.

Antimicrobial activity

The antibacterial activity of the compound was carried out by the disc diffusion method using nutrient agar medium[16]. Gram-positive and gram-negative bacteria species were collected as pure cultures from the Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka-1344. The sterile Whatman-1 5.0 mm filter paper discs, impregnated with 50 µg of compound and ciprofloxacin 5 µg, were placed gently on the previously marked zones on the agar plates. The plates were incubated at 37°C for 24 h. The water discs were used as a negative control to minimize the effect of filter paper discs against test organisms. The zones of inhibition (mm) produced by the compound and ciprofloxacin against different microorganisms were measured.

Conclusion

Cinnamic acid derivatives are naturally occurring substances found in different plants. In the literature review, cinnamic acid and its derivatives revealed significant biological activities such as antioxidant, cytotoxic, antidiabetic and antimicrobial, *etc*. The compound isolated from these plant *p*-pentadecayl ethyl cinnamate possess lower activity compared to the other derivatives and standards. It may be due to the presence of a long aliphatic chain in the benzene ring. Further study required for isolation of potential active components from GVM and identify the components

Acknowledgments

The authors are grateful to the Department of Pharmacy Gono Bishwabidyalay, Savar, Dhaka-1344 and Wazed Miah Science Research Centre, Jahnagirnagar University, Savar, Dhaka for the laboratory facility to conduct the research work.

References

1 Wink M, *Medicines*, 2 (2015) 251.
2 Khan M A & Khan J, *National Health Portal (NHP),* India, (2016) 14.
3 Vijaya T, Ramarao N V, Babu A N, Kumar M S, Nirojini P S, Reddy B S & Nadendla R, *Int J Biol Pharm Res*, 4 (2013) 878.
4 Tropical Plants Database, Ken Fern, https://tropical.theferns.-info/viewtropical.php?id=Glochidion%20velutinum.
5 Deb J, Saha S, Deb N K, *Int J Herb Med* 7 (2019) 03.
6 Gong X, Li Y & Qu H, *Molecules,* 19 (2014) 18705.
7 Chetty M, Sivaji K S K & Rao K T, *Flowering Plants of Chittoor Dist A P* (Students Offset Printers, Tirupati, India), 2008, 317.
8 Are P C, Ram R, Reddy A & Puchhakayala G, *Eur J Biol Sci,* 3 (2011) 126.
9 Ramarao N V, Nadndla R, Arunkumar M, Chowdary B S, Reddy B S, Nirojini P S & Begum S S, *Int J Phytopharm,* 4 (2013) 311.
10 Sandhya S, Chaitanya R S N A K K, Vinod K R, Rao K N V, Banji D, Sudhakar K & Swetha R, *Arch Appl Sci Res*, 2 (2010) 309.
11 Sandhya S, Chaitanya R S N A K K, Vinod K R, Chandrasekhar J & Banji D, *Eur J Biol Sci,* 3 (2011) 78.
12 Sandhya S, Chaitanya R S N A K K, Banji D & Aradhana, *J Glob Trends Pharm Sci*, 2 (2011) 91.
13 Hossain M S, Das A K, Rabbi F & Ahmad M, *J Bangladesh Acad Sci,* 41 (2017) 247.
14 Karuppusamy S & Rajasekaran K M, *Global J Pharmacology*, 3 (2009) 63.
15 Prieto P, Pineda M & Aguilar M, *Anal Biochem*, 269 (1999) 337.
16 Braca A, Tommasi N D, Bari L D, Pizza C, Polit M & Morelli I, *J Nat Prod,* 64 (2001) 892.
17 Bauer A W, M D, Kirby W M M, Sherris J C & Truck M, *Am J Clin Pathol,* 45 (1966) 493.