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Isolation and characterization of new potential allelochemical from *Bidens biternata* (Lour.) Merrill & Sherff

Vijayshri Surywanshi* and R. N. Yadava

Natural Products Laboratory, Department of Chemistry, Dr. Hari Singh Gour Vishwavidyalaya (A Central University) Sagar (M.P.), India

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ABSTRACT

A new potential allelochemical A,m.f. $C_{33}H_{40}O_{20}$, $[M^+]$ 756, mp.228-231^{0}C has been isolated from ethanolic extract of the stems of Bidens biternata(Lour.) Merrill &Sherff. Along with two known compounds B and C. The structure of the compound A was characterized as 5, 7, 8, 4' tetra hydroxy 3, 5' di-methoxy flavone- 7-O-α-L-rahmnopranosyl-4'-O-β-D-arabinopyranosyl(1→4)-O-β-D-xylopyranoside by various colour reactions, spectral analysis and chemical degradations.

Keywords: *Bidens biternata* (Lour.) Merrill &Sherff, Compositae, Stems, Allelochemical.

INTRODUCTION

Bidens biternata(Lour.) Merrill & Sherff[1-4] belongs to family Asteraceae which is commonly known as "chirchitta" in Hindi. This plant is a native of America[5].It is a less common weed, grows on hill slopes and in lateritic or sandy soil. It is mostly found throughout in India ascending to 1000 m in Himalaya, China, japan, S.E. Asia, Australia and Africa[6]. It is a rich source of bioactive compounds sach as polyacetylenic glycosides, aurons, aurons glycosides, p-coumeric acid derivatives, flavonoids, flavonoid glycosides and sesquiterpenes. This plant is used as a leafy vegetable by the paniya and kattunaayila tribes of Waynadu Districts in Kerela. Extracts from this herb are applied in leprosy and skin diseases also useful in fistulae, pustules, tumours hepatitis, cold, cough and dysentery. Its juice of leaves applied to heal ulcers and to cure eye and ear complaints. Earlier workers[7-11] have reported various chemical constituents from these plants. In the present paper we reported on isolation and structure elucidation of new allelochemical 5, 7, 8, 4' tetra hydroxy 3, 5' di-methoxy flavone- 7-O-α-L-rahmnopranosyl-4'-O-β-D-arabinopyranosyl(1→4)-O-β-D-xylopyranoside (A) along with two known compounds Luteolin (B), Apigenin (C) from ethanolic extract of stems of this plants.

EXPERIMENTAL SECTION

All of the melting point were determined on a thermo electrical melting points apparatus and are uncorrected. The IR spectra were recorded in KBr disc on FT-IR spectrophotometer, Shimadzu 8400S. UV spectra were recorded on Systronics-2201 UV/Vis Double Beam spectrophotometer. The NMR spectral data were obtained on Bruker DRX (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) in DMSO-d_6 with tetramethylsilane as internal standard. The FABMS was recorded on Jeol - SX (102) mass spectrometer.

Plant Material

The stems of the plants were collected locally around Sagar region and were taxonomically authenticated by Taxonomist, Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P.) India. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry of this university.

Extraction and Isolation

Dried and powdered stems of this plant (3.5 kg) were extracted with ethanol in a Soxhlet apparatus for 74 h. The ethanolic extract of stem of the plant was further successively partitioned with chloroform, ethyl acetate, acetone and methanol. The acetone soluble fraction was further concentrated under reduced pressure to give brown viscous mass (2.35 gm), which was subjected to TLC examination using nBAW (4:1:5) as solvent and I_2 vapours as visualizing agent. It gave three spots indicating it to be mixture of three compounds A, B and C. These compounds were separated by TLC and purified by column chromatography over silica gel using $CHCl_3$:MeOH (4:8) as eluents and studied separately.

Study of Compound A

It was analyzed from methanol to give 1.20 gm. It has mp.228-230^{0}C,m.f. $C_{33}H_{40}O_{20}$, [M^+] 756 (FABMS); found (%): C 52.63, H 5.19, O 42.22 calcd. $C_{34}H_{42}O_{21}$: C 52.00, H 5.27, O 42.36; UVλ_{max}(nm):(MeOH) 228,287, 293, 326, (+$AlCl_3$) 294, 318, 346, 382. IR (KBr) υ_{max}3409,1734, 1658, 1460, 1040, 1278, 840, 780. ^{1}H NMR (300MHz DMSO-d_6)δ (ppm); 6.84 (1H,s , H-6), 12.47 (1H, s, 5-OH), 12.65 (1H, s, 7-OH), 12.59 (1H, s, 8-OH), 3.90(3H, s, 3-OCH_3), 8.04(1H,d , *J* 2.3 Hz H-2', H-6'), 7.91(1H, d , *J* 8.9 Hz H-3'), 9.97 (1H, s, 4'-OH), 3.84(3H, s, 5'-OCH_3), 5.28 (1H, d, *J* 1.2 Hz H-1"), 3.38-3.79 (4H, m, H-2", H-3", H-4", H-5"),0.97 (3H, d, *J* 6.2 6"-OCH_3) 5.12 (1H, d, *J* 6.9Hz, H-1'"), 3.26-3.72 (3H, m, H-2'", H-3'", H-4'" H-5'"),5.57 (1H, d, *J* 5.8 Hz, H-1""), 3.74-4.10 (4H, m, H-2"", H-3"", H-4"", H-5""). ^{13}C NMR (75 MHz, DMSO-d_6) δ(ppm):151.9 (C-2), 144.1(C-3), 175.8 (C-4), 148.3(C-5), 131.9 (C-6),146.2 (C-7), 129.3 (C-8), 152.1 (C-9), 123.6 (C-1') 113.9 (C-2') ,145.8(C-3'), 152.1(C-4'),113.1 (C-5'),118.2 (C-6'), 61.4 (3-OCH_3), 58.3 (5'-OCH_3), 104.8 (C-1"), 72.5(C-2"), 72.8(C-3"), 72.9(C-4"), 72.4(C-5"), 18.3(C-6") 107.2 (C-1'"), 74.8 (C-2'"), 77.3 (C-3'"),66.8 (C-4'"),65.9 (C-5'"), 101.9 (C-1""), 76.3 (C-2""), 74.2 (C-3""),67.5 (C-4""),66.4 (C-5"").

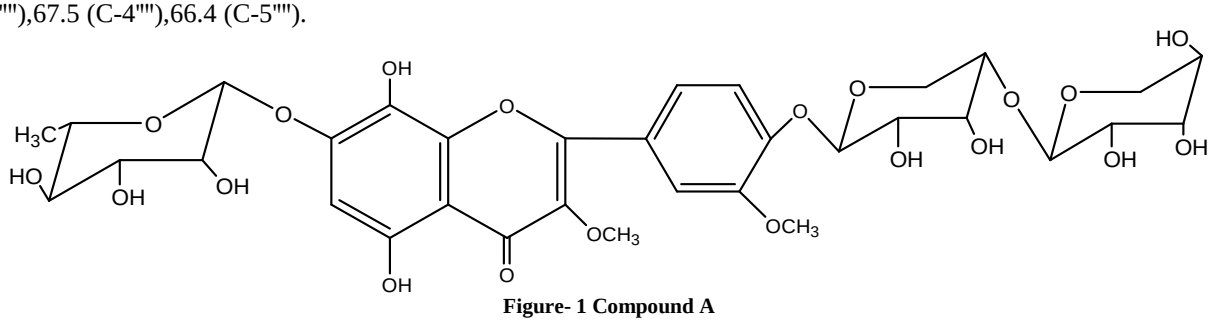

Figure- 1 Compound A

Acid Hydrolysis of Compound A

250 mg of the compound was dissolved in ethanol (25ml) and refluxed with 40ml of H_2SO_4 on water bath for 5-6 h. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether (Et_2O). The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$: MeOH (4:8) to give compound A-1 identified as 5, 7, 8, 4' tri hydroxy 3, 5' di methoxy flavone. The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) solvent and aniline hydrogen phthalate as spraying reagent, showed the presence of L- rhamnose (R_f0.38), D-xylose (R_f0.26), D- arabinose (R_f0.23).

Study of Compound A-1

It was analyzed from methanol to give 950 mg. It has m.p. 221-223 ^{0}C, m.f. $C_{17}H_{14}O_8$, [M^+] 346 (FABMS); found (%): C 59.23, H 4.09, O 37.12 calcd. $C_{34}H_{42}O_{21}$: C 59.14, H 4.12, O 37.15; UVλ_{max}(nm):(MeOH) 339, 258, 273 (+$AlCl_3$) 379. IR (KBr) υ_{max} 3476,1632, 1605, 1493, 988. ^{1}H NMR (300MHz, DMSO-d_6)δ (ppm); 6.82 (1H, s, H-6), 12.43 (1H, s, 5-OH), 12.68 (1H, s, 7-OH), 12.55 (1H, s, 8-OH), 3.92(3H, s, 3-OCH_3), 7.43(1H,d , *J* 2.3 Hz H-2', H-6'), 6.83(1H, d , *J* 8.9 Hz H-3'),12.67 (1H, s, 4'-OH), 57.3(3H, s, 5'-OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ(ppm) :152.9 (C-2), 144.5(C-3), 174.6 (C-4), 146.2(C-5), 130.3 (C-6),146.8 (C-7) ,128.3 (C-8), 152.7 (C-9), 112.7 (C-2') ,144.7(C-3'), 153.6(C-4'),112.6 (C-5'),117.2 (C-6'), 62.4 (3-OCH_3), 59.3 (5'-OCH_3).

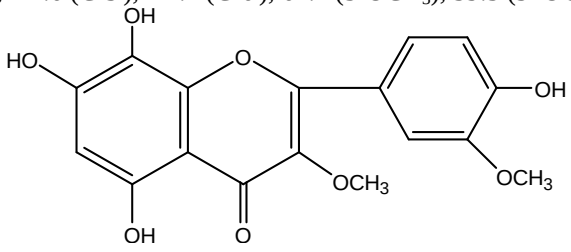

Figure- 2 Compound A-1

Per methylation of Compound A

Compound A (25 mg) was refluxed with MeI (15 ml) and Ag_2O (10ml) in DMF (20mg) for two days and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 7-8 h, to give methylated aglycone identified as 7, 4',-dihydroxy-3, 5, 8, 5' tetramethoxy flavone and methylated sugars were identified as 2, 3, 4- tri- O-methyl- L-rhamnose (R_G 1.03), 2, 4- di-O-methyl- D- arabinose (R_G 0.63), 2, 3- di-O-methyl-D- xylose (R_G 0.76).

Enzymatic Hydrolysis of Compound A

Compound 35 mg. was dissolved in MeOH (15ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 2 days and filtered. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent, which showed the presence of L- rhamnose (R_G 1.02), (Co-PC). The proaglycone was dissolved in MeOH (16ml) and further hydrolysed with equal volume of almond emulsion yielded D- arabinose (R_G0.65), D- xylose (R_G0.66) and aglycone, identified as5, 7, 8, 4' tetra hydroxy 3, 5' di-methoxy flavone.

Study of Known Compound B

It was crystallized from acetone to give 365 mg.It has m.f. $C_{15}H_{10}O_6$, m.p. 323-325^{0}C, [M^+]286(EMIS) found (%): C 62.89, H 3.51, O 33.54 calcd (%) for m.f. $C_{15}H_{10}O_6$ C 62.93, H 3.49, O 33.56 UVλ_{max}(nm):(MeOH) 206, 257, 265, 346 nm. IR (KBr) υ_{max} 3426, 2921, 1656, 1619, 1504, 1363, 1257, 1165, 1036, 836 cm^{-1}. ^{1}HNMR (300MHz DMSO-d_6)δ (ppm);6.95 (1H, s, H-3),6.24 (1H, d, *J* 2.2 Hz, H-6),6.83 (1H,d , *J* 2.2 Hz, H-8), 12.4(1H, s, 5-OH), 10.62(1H, s, 7-OH), 7.35(1H, d, *J* 2.0 Hz, H-2'), 6.98(1H, d, *J* 8.2 Hz,H-5'),7.48 (1H, d, *J* 8.1 Hz, H-6'),9.92(1H, s, 3'-OH), 9.63(1H, s, 4'-OH), ^{13}CNMR (75MHz, DMSO-d_6), δ(ppm), : 168.2 (C-2), 105.4(C-3), 182.5 (C-4), 162.3(C-5), 98.5 (C-6),165.3 (C-7) ,92.3 (C-8),258.3 (C-9), 104.2 (C-10),121.5 (C-1'),116.9 (C-2') ,146.3(C-3'), 147.3(C-4'),116.8 (C-5'),121.4 (C-6'). Then it was identified as Luteolin by comparison of its spectral data with reported literature values[12].

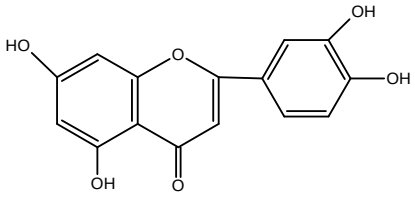

Figure- 3 Compound B

Study of Known Compound C

It was crystallized from acetone to give 290mg.It has m.f. $C_{15}H_{10}O_5$, m.p.327-329^{0}C[M^+]270(EMIS) found (%): C 66.69, H 3.72, O 29.67 calcd (%) for m.f. $C_{15}H_{10}O_6$ C 66.66, H 3.70, O 29.70 UVλ_{max}(nm):(MeOH) 268, 332 (+NaOMe) 272, 382 (+$AlCl_3$) 274, 300, 349, 386. IR (KBr) υ_{max} 3434, 1659, 1621, 1086, 836 . ^{1}HNMR (300MHz DMSO-d_6)δ (ppm);6.69 (1H, s, H-3),6.63 (1H, d, *J* 2.2 Hz, H-6),6.73 (1H,d , *J* 2.2 Hz, H-8), 11.4(1H, s, 5-OH),10.64(1H, s, 7-OH), 7.94(1H, d, *J* 2.0 Hz, H-2'and H-6'), 6.96(1H, d, *J* 8.2 Hz,H-3' and H-5'),9.65(1H, s, 4'-OH), ^{13}CNMR (75MHz, DMSO-d_6), δ(ppm), : 164.2 (C-2), 104.3(C-3), 182.6 (C-4), 162.1(C-5), 100 .5 (C-6), 163.5(C-7) ,98.3 (C-8), 157.3(C-9), 106.2(C-10),122.5 (C-1'),128.5 (C-2' and C-6') ,116.3(C-3' and C-5'), 161.8(C-4'). Then it was identified as Apigenin by comparison of its spectral data with reported literature values[13].

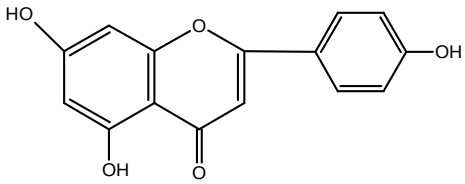

Figure- 4 Compound C

RESULTS AND DISCUSSION

A new allelochemical **A** has been isolated from acetone soluble fraction of ethenolic extract of the stems of this plant. Compound **A** has molecular formula $C_{33}H_{40}O_{20}$ mp.228-231^{0}C, [M^+] 756 (FABMS). It gave Molisch and Shinoda tests[14] showing its flavonoidalglycosidic nature. Its IR spectra showed strong absorption bands at 3478, 1636, 1608, 1498 and 982 cm^{-1}. In UV spectrum two bands at 267 and 361 nm showed its flavonoidal skeleton. The bathochromic shift of 7 nm with MeOH and 40 nm $AlCl_3$ relative to methanol showed the presence of OH groups at C-8 and C-5 position in the aglycone A-1.

In ^{1}H NMR spectrum of compound 1 three singlets at δ12.47,δ12.65 and δ12.59 confirmed the present of OH groups at C-5, C-7 and C-8 position respectively. A singlet at 3.90 confirmed the present of OMe group at C-3 position. A singlet at 12.68 confirmed the present of OH group at C-4' position. A singlet at 59.3 confirmed the present of OMe group at C-5'position. In ^{1}HNMR spectrum of the agylcone A-1 a singlet at δ6.82 was assigned to H-6 of ring A.A doublet at δ7.43 was assigned to H-2 ' H-6 ' in ring B. A doublet at δ6.83 was assigned to H-3' in ring B. The anomeric proton at δ5.28 (1H, d, *J* 1.2 Hz), 5.12 (1H, d, *J* 6.9 Hz) and δ5.57 (1H, d, *J* 5.8 Hz), were assigned for H-1", H-1'", H-1'""of L- rhamnose, D- Xylose and D- arabinose, respectively.

In the mass spectrum of compound A, characteristic ion peaks at *m/z* 756 [M^+], 610 [M^+-L- rhamnose], 478 [M^+-D-arabinose], and 346 [M^+- D- xylose aglycone] were found by subsequent losses from the molecular ion of each molecule of L- rhamnose, D- arabinose, and D- xylose showing D- arabinose was terminal sugar, of L- rhamnose was linked to aglycone at C-7 position and D- xylose was attached at C-4' position of aglycone.

Acid hydrolysis of compound A with 10% ethanolic H_2SO_4 gave aglyconeA-1 m.p. 224-227 ^{0}C, m.f. $C_{17}H_{14}O_8$ [M^+] 346 (EIMS) and sugar moiety (ies). These were separated and studied separately. The aglycone**A-1** was identified as 5, 7, 8, 4' tetra hydroxy 3, 5' di methoxy flavone (see in Experimental section).

The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated subjected to paper chromatography examination and sugars were identified as L-rhamnose(R_f 0.38), D- arabinose (R_f 0.23), and D- xylose (R_f 0.26) (Co-PC)[15] Periodate oxidation of compound A, confirmed that all the sugars were present in the pyranose form[16].

The position of sugar moieties in compound A were determined by permethylation followed by acid hydrolysis yielded methylated aglycone identified as 7, 4',-dihydroxy-3, 5, 8, 5' tetramethoxy flavone showed that glycosidation was involved at 7, 4' position of the aglycone and methylated sugars were identified as 2, 3, 4- tri- O-methyl- L-rhamnose (R_G1.03), 2, 4- di-O-methyl- D- arabinose (R_G0.63), 2, 3-di-O-methyl-D-xylose (R_G 0.76) indicating that C-1"-OH of L- rhamnose was linked to C-7 position of the aglycone. C-1 '"-OH of D-arabinose was linked to C-4'" OH of D-xylose and C-1'" OH of D-xylose was attached with C-4' position of the aglycone. therefore it was concluded that interlinkage (1→4) was found between D- arabinose and D-xylose which was further confirmed by ^{13}C-NMR spectra (see in experimental section).

Enzymatic hydrolysis of compound A with takadiastase enzyme liberated L- rhamnose(R_f 0.38) and proaglycone identified as 5, 7, 8, 4' tetra hydroxy 3, 5' di methoxy flavone-4'-O-β-D-arabinopyranosyl(1→4)-O-β-D-xylopyranoside showed the presence of α linkage between L- rhamnose andProaglycone on further hydrolysis with almond emulsion enzyme liberated D- arabinose followed by D-xylose and aglycone suggesting the presence of β linkage between D- arabinose and D-xylose as well as between D-xylose and aglycone.

On the basis of above evidences the structure of compound A was characterized as 5, 7, 8, 4' tetra hydroxy 3, 5' di methoxy flavone- 7-O-α-L-rahmnopranosyl-4'-O-β-D-arabinopyranosyl(1→4)-O-β-D-xylopyranoside.

CONCLUSION

The phytochemical analysis of ethanolic extract of *Bidens biternata* (Lour.) Merrill &Sherff shown the presence of new allelochemicals. The result suggested the medicinal importance of the plant.

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