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Chemical Investigation of Merremia Gangetica

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Abstract : ***Three flavonoids Apigenin, Apigenin 7-O-methylether, Apigenin 7-O-β-D-glucopyranoside and a phenolic acid-Chlorogenic acid have been isolated from the aerial parts of Merremia gangetica. Detailed spectral techniques including UV, IR, ^{1}H NMR, ^{13}C NMR, positive ESIMS data have been provided for the above compounds.***

Keywords: Merremia gangetica: phenolic acid, chlorogenic acid

Introduction *Merremia gangetica* is twining (or) prostate herb. It is a pan trophical species found in India and recorded as medicinal plant. (Chatterjee et al., 1995). The Ayurvedic practitioners consider a decoction of the plant to act as deobstruent, and diuretic. A decoction is also used for treating rheumatism, neuralgia and headache, diseases of kidney, bladder, heart and lungs, fevers, strangury, urethral discharges, anaemia, leukoderma and for resolving tumours as well. The leaf juice of the plant is prescribed for migraine and as an ear drop to get relief from abscesses and ulcers. The powdered leaves as a snuff during epileptic seizures. (Cook, 1996; Babu et al., 2009; Dave et al., 2004; Pratap et al., 2010; Rajasab et al., 2004).

In the absence of any work on a systematic chemical investigation of the aerial parts of this plant and the medicinal properties reported, the plant was taken up for systematic chemical investigation and the results leading to the isolation and characterization of a phenolic acid-chlorogenic acid and three flavonoids-Apigenin, Apigenin 7-O-methylether, Apigenin 7-O-β-D-glucopyranoside are presented here.

Fig.1: Compound (1)

Fig.2: Compound (2) R=H; Compound (3) R=O CH_3; Compound (4) R=β-D-glucopyranosyl

Experimental:

Plant material

Fresh aerial parts were collected from karasur village, puducherry on may 2013 and authenticated by the Dept. of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry. Where a voucher specimen was deposited.

Extraction and Isolation

The air dried aerial parts of the plant were extracted thrice with boiling 95% EtOH (3X5L) and concentrated in vacuo to 500ml. The aqueous alcoholic concentrate was fractionated into Benzene, Ether, Ethyl acetate, Ethyl methyl ketone solubles. Benzene, fraction on PC gave (15% AcOH) no characteristic spots for polyphenolics and was not workedup further. The ether fraction was column chromatographed over sephadexLH-20. 20 fractions each of 10 ml were collected.

Compound (1) (30 mg) from fractions 1-4 and compound (2) (40 mg) from fractions 5-12 were obtained.

The Ethyl acetate and Ethyl methyl ketone fractions were found to be identical on PC (15% AcOH). Hence mixed together and coloumn chromatographed on Sephadex LH-20. 19 fractions each of 10 ml were collected. Compound (3) (10 mg) from fractions 6-13 and compound (4) (25 mg) from fractions 14-19 were obtained.

a) Chlorogenic acid (1)

Colourless needles (Me_2Co), $C_{16}H_{18}O_9$, mp. 199-200℃; $[\alpha]^{16}_D$-35.2° (H_2O) blue fluorescence under UV changing yellow green with UV/NH_3 ; UV (λ max., nm) (MeOH): 243,305 sh, 329; ($AlCl_3$) 260 sh, 310, 358; ($AlCl_3$/HCl) 240, 300 sh, 327, (NaOMe) 260 sh, 300 sh, 380; ^{1}H NMR 350 MHz) DMSO-d_6 δ ppm): 9.64, 9.22, (each brs, each 1H, OH-7', 8'), 7.41 (d, J = 16Hz, 1H, H-3'), 7.39 (d, J =2Hz, 1H, H-9), 7.00 (dd, J=8 & 2 Hz, 1H, H-5'), 6.65 (d, J= 8Hz, 1H, H-6'),6.15(d J=16 Hz, 1H,H-2') 5.05 (m,1H,H-3), 4.95,4.79,3.92 (each brs, each 1H,1,4,5-OH). 3.9-3.6 (m, 2H, H-4,5), 2.05-1.73 (m,4H,2,6-CH_2). ^{13}C NMR(67.89 MHz, broad band decoupled, DMSO-d_6 δ ppm). 175.00 (C-7), 166.13 (C-1),148.21 (C-7') 145.92 (C-6') 145.08 (C-3'), 125.68 (C-4'), 121.62 (C-9'), 115.82 (C-2') 114.49-(C-5'/8'), 114.32 (C-5/8') 73.72 (C-1), 70.74 (C-3), 70.62 (C-4), 68.40 (C-4), 68.40 (C-5) 36.95 (C-2) 36.45 (C-6): MS/(EIMS, m/z Intensity as %) 354 (M+, 5), 336 (M-H_2O,32),180 (Caffeic acid $^+$, 60) 163 (caffeoyl, 100) 162 (180-H_2O,23).

b) Apigenin (2)

Light yellow needles, (MeOH-Me_2Co) mp 348-350 ℃ purple under UV changing to yellow with UV/NH_3; UV (λ max., nm) (MeOH): 267, 296 sh, 336; (+NaOMe) 275,324,392; (+NaOAc) 274,301,376; (+NaOAc/H_3BO_3) 268, 302 sh, 338; (+$AlCl_3$) 276, 301, 348, 384, (+$AlCl_3$/HCl) 276,299,340,381; ^{1}H NMR (200.13 MHz, DMSO-d_6): 13.39 (1H,s,OH-5),10.98(1H,s,OH-7) 8.34(2H,d, J,7.8 Hz, H-2', H-6') 7.34 (2H,J 7.9 Hz H-3' H-5') 7.20 (1H,s,H-3), 6.89 (1H d, J,2.4 Hz, H-8) 6.60(1H,d,J,2.45 Hz H-6); ^{13}C NMR (50.32 MHz, DMSO-d_6): 181.8 (s,C-4), 164.3 (s,C-7),163.8 (s,C-4), 161.39(s,C-5), 157.34(s,C-9),128.5(d,C-2,C-6),121.2 (s,C-1), 116.0 (d,C-3,C-5), 103.7 (s,C-10), 102.9 (s,C-3),98.9(s,C-6),94.0(s,C-8); ESIMS: m/z (rel.int. as %) 271 ($M+H^+$, 100).

c) Apigein 7-O-methyl ether (3)

Yellow needles, (EtOAc-petrol) mp 325-327 ℃ purple under UV and yellow under UV/NH_3. UV (λmax., nm) (MeOH): 268,293,326(+NaOMe)275,324,384(+NaOAc)260,301,370; (+NaOAc/H_3BO_3) 268,293,326; (+$AlCl_3$) 276,301,345,382; (+$AlCl_3$/HCl) 276,294,340,381 ESIMS: m/z (rel.int. as %) 285($M+H^+$, 100).

d) Apigein - 7-O-β-D-glucopyranoside (4)

Pale yellow needles, (MeOH-Me_2CO) mp. 251-253 ℃ UV (λ max., nm) (MeOH): 268,333; (+NaOMe) 245 sh 269, 301 sh, 386; (+NaOAc) 256 sh,267,355,386; (+NaOAc/H_3BO_3) 267,340; (+$AlCl_3$) 276,300,348,386; (+$AlCl_3$/HCl)277,299,341,382; ^{1}H NMR (400 MHz, DMSO- d_6) 7.9(2H J 8.7, H-2', H-6') 6.9 (2H,J 8.9 Hz,H-3',H-5'),6.8 (1H,d,J 2.5 Hz,H-8) 6.7 (1H,s,H-3)6.4. (1H,d,J 2.4 Hz,H-6) of aglycone; 5.05 (d-J-7.25, Hz,H-1") 4.45 (d,J 11Hz,H-6 α),4.15(d,J 11.5Hz,H-6β), 3.85(m,H-5"),3.50(m,H-4"),3.45(m,H-3" H-2") of glucose: ESIMS: m/z (rel.int. as %) 455 ($M+Na^+$,100).

Results and Discussion

From the alcholic extract of air dried aerial parts of *Merremia gangetica* one phenolic acid and three flavonoids were isolated.

Compound (1)

Compound (1) gave deep blue with Fe^{3+}, rosy red with phenol reagent, brisk effervescence with saturated $NaHCO_3$, blue flurescence under UV changing to yellow green under UV NH_3.It had λ max (MeOH) 243, 305sh and 329 nm suggesting it to be phenyl proponoid. Alkali hydrolysis yielded caffeic acid along with an aliphatic acid. EIMS exhibited molecular ion peak at m/z 354 along with other fragment ions at m/z 336, 180 and 163. The peak at m/z 180 was due to caffeic acid thereby suggesting it to be a caffeoyl ester of quinic acid. The fragmentation pattern was found to be in close agreement with reported values. (Sakushima et al., 1985). This was further supported by HPLC, ^{1} H NMR and ^{13}C NMR spectrum. Thus the compound (1) was identified as 3-O-caffeoyl quinic acid:chlorogenic acid. The identity was further confirmed by Co-PC with an authentic sample from Berberis aristata (Nair et al., 1991).

Compound (2)

Compound (2) was purple under UV and light yellow under UV/NH_3 and had Rf values characterstic of a flavone. The presence of 5,7,4' free OH groups was indicated by UV spectral analysis. The presence of aromatic protons at 6, 8, 2', 6' and 3' 5' were confirmed by ^{1}H NMR signals. This was further supported by ^{13}C NMR spectral data. Thus the structure of flavone was established 5,7,4' trihydroxy flavone apigenin (Sane et al., 1995; Jhou, et al., 1993; Qian-cutrone et al., 1996).

Compound (3)

Compound (3) had fluorescence and λmax similar to that of compound (2).The UV spectral analysis with shift reagent NaOAc revealed the absence of free 7-OH. On demethylation

with KI it gave compound (2). Thus the structure of compound (3) was established as apigenin7-O-methylether.

Compound (4)

Compound (4) gave pink colour with Mg/HCl, positive Molisch's test and was purple under UV and yellow under UV/NH_3. On acid hydrolysis yielded and an aglycone identified as apigenin and sugar as D-glucose by co-chromatography. The comparison of band II of NaOAc uv spectrum to methanol spectrum suggested that the 7-OH was involved in glycosylation (Obatomi et al., 1994; Gunasekara et al., 1995). In addition the ^{1}H NMR exhibited signals characterstic of a flavone glycoside. The anomeric proton of sugar appeared at δ,5.05. The mass spectrum exhibited peak at m/z 455 ($M+Na^+$, 100) expected for the molecular formula $C_{21}H_{20}O_{10}$. Thus the compound (4) was characterized as Apigenin 7-O-β-D-glucopyranoside.

Conclusion

In the present study we report the isolation and characterization of a phenolic acid and three flavonoids from *Merremia gangetica* of *convolvulaceae.* All these compounds were isolated and characterized for the first time from this *species*. The results of primary assay revealed that the crude extract, the flavonoids and the phenolic acid from this plant could be used for cough, headache, neuralgia and rheumatism. This research assumes significant as the Western population looking for the natural remedies which are safe and effective comparing to the adverse effects of the synthetic drugs.

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