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Phytochemical analysis of aerial Parts of Ixora paviflora

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Abstract : *Ixora paviflora* (Rubiaceae) a small evergreen tree or shrub found throughout the greater part of India. The fruits and roots are used as antidote. The flowers are pounded in milk and given for relieving whooping cough while the decoction of bark is used for anemia and debility. Chemical analysis of aerial parts of *Ixora paviflora* afforded β-sitosterol, β-sitosterol-β-D-glucoside, kaempferol and kaempferol-7-O-methyl ether. The structures of these compounds were determined by extensive use of IR, UV, NMR spectroscopy.
Keywords: *Ixora paviflora,* β-sitosterol, , β-sitosterol-β-D-glucoside, kaempferol and kaempferol-7-O-methyl ether.

Introduction

Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed (1). Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations are based on plants (2).
However, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants. The use of medicines from plants in the form of local medicine dates back to 4000-5000 B.C. While the medicinal values of these plants are due to the presence of small doses of active compounds which produces physiological actions in the human and animal body It was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries (3,4).

Ixora paviflora (Rubicaceae) is a small, much branched evergreen tree or shrub found throughout the greater part of India, from the gangetic plain eastwards to Assam and southwards to Kerala and in Nicobar Islands; it is also grown in the gardens. Leaves opposites, sub sessile, oblong, elliptic or ovate oblong, 3 to 6 inches long, coriaceous, glaucous; flower in larger corymbose terminal cymes, small, numerous, white or pinkish, fragrant, berries black, globose, 0.25

inches in diameter, somewhat didymous, seed planoconvex(5). A new flavone glycoside isolated from the stem of *Ixora paviflora* (6).The present investigation deals with the isolation and identification of as β-sitosterol,β-sitosterol-β-D-glucoside, kaemp - ferol and kaempferol-7-O-methyl ether from the aerial parts *Ixora paviflora*

MATERIAL AND METHOD

Plant Material

The aerial parts of *Ixora paviflora* were collected from Forest Research Institute Campus, New Forest, Dehradun, Uttarakhand in Feb, 2009, The plant species were identified by Dr. Sumer Chand, Systematic Botany Department, FRI, Deharadun, Uttarakhand, India.

Extraction and Isolation

The air-dried and powdered aerial parts (1.5 kg) were exhaustively defatted with light petroleum ether (60-80^0). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (10 g). The aqueous layer was concentrated under reduced pressure and then partitioned with ethyl acetate and 50% aqueous methanol. The ethyl acetate fraction was dried under reduced pressure to give EtOAc extract (8.03 g). The aqueous methanol extract was concentrated under reduced pressure to give methanol extract (12.5 g). The chloroform, ethyl acetate and methanol extracts were column chromatographed over various adsorbent with various solvent.

The chloroform extract (5.0 g) was column chromatographed over Si-gel using gradient elution with C_6-H_6-EtOAc (10:0→9:1) afforded compound 1 (145mg), and compound 2 (105 mg), and various other fractions. The fractions with the same components (monitored by TLC) were mixed, dried and chromatographed over Si-gel eluted with $CHCl_3$:MeOH (98:2) afforded compound 3 (40 mg) and compound 4 (65 mg).

RESULT AND DISCUSSION

COMPOUND:1

It was crystallized from methanol as white needles, M.P. 135-137^{0}C.

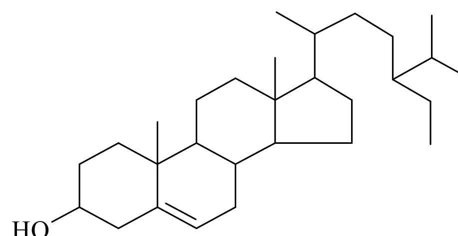
$[\alpha]_D^{25}$: -36 (c=0.1, $CHCl_3$).

Elemental Analysis: Found values C=84.1%, H=12.08%, required values for $C_{29}H_{50}O$; C=84.05%, H=12.07%, Molecular weight 414.

IR (ν_{max}^{KBr}): 3340, 2970, 2959, 2920, 1463 cm^{-1}.

Elemental analysis of compound **1** corresponded to the molecular formula $C_{29}H_{50}O$. It was found to be sterol as it responded positive to Liberman and Nollers' tests. It also shows positive TNM test for unsaturation. It was soluble in petroleum ether, C_6H_6, $CHCl_3$ and in hot MeOH but insoluble in water and alkali. Its negative test with Molisch's reagent indicates the non-glycosidic nature of the compound. The IR spectrum of compound **1** showed characteristic absorption at 3340 cm^{-1} for OH group, 2970, 2959, 2920 for C-H stretching and 1640 cm-1 for C=C stretching.

On the basis of these experimental data compound **1** was identified as β-sitosterol. It was further confirmed by co-TLC, co-IR and MMP with that of the authentic sample (7).



COMPOUND: 2

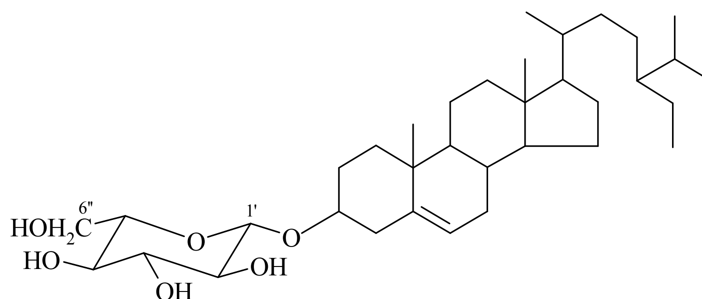
It was crystallized from MeOH as white crystals, M.P. 289-291°C.

$[\alpha]_D^{25}$: -39 (c=0.9, C_5H_5N).

Elemental Analysis: Found values C=72.68%, H=10.2%; required values for $C_{35}H_{60}O_5$; C=72.97%, H=10.41%; Molecular weight 576.

IR (ν_{max}^{KBr}): cm^{-1} 3400, 1640, 878 and 780.

Hydrolysis of Compound 2: Hydrolysis of compound **2** was carried out by refluxing 5 mg of the compound with 5% HCl for 7 hr on water bath. The contents were cooled and extracted with chloroform where a crystalline compound as white needles was obtained. It was identified as β-sitosterol (compound **1**). The sugar was identified as glucose by PC.



Compound **2** gave positive Molisch's test and did not reduce Fehling,s solution. It also responded to LB test, characteristic for sterols and positive TNM test for unsaturation. The IR spectrum showed characteristic absorption at 3400 (OH stretching) and 1640 (C=C stretching), 878 and 780 cm^{-1} (of glycoside) (8). On acid hydrolysis compound **2** afforded an aglycone (M.P. 135-136°C), identified as β-sitosterol by co-TLC, co-IR and MMP with an authentic sample. The sugar was identified as glucose by co-PC with an authentic sample. As the compound did not reduce Fehling's solution, it indicated that the reducing group of sugar was involved in the glycosidic linkage.

On the basis of above observations compound **2** was identified as β-sitosterol-β-D-glycoside. It was further confirmed by co-TLC and co-lR and MMP with an authentic sample.

COMPOUND: 3

It was crystallized from MeOH as yellow needles, M.P. 222-223°C.

Elemental Analysis: Found values C=62.69%, H=3.17%, required values for $C_{15}H_{10}O_6$; C=62.93%, H=3.49%; Molecular weight 286.

IR (v_{max}^{KBr}): cm^{-1} 3480, 3260, 1645, 1610, 1510, 1415, 1345, 1285, 1220, etc.

EI-MS: m/z 286 $[M]^+$, 257, 229, 176, 165, 153, 133, 105, 77.

^{1}H-NMR (300 MHz, CD_3OD): δ 6.13 (1H, *d*, *J*=2.5 Hz, H-6), 6.44 (1H, *d*, *J*=2.5 Hz, H-8), 6.81 (2H, *d*, *J*=8.7 Hz, H-13, 15), and 8.09 (2H, *d*, *J*=8.7 Hz, H-12, 16) .

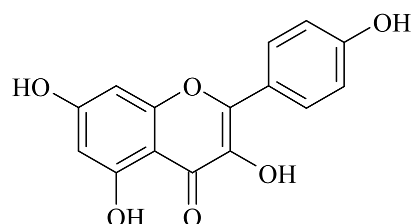
^{13}C-NMR (75 MHz, CD_3OD): δ 148.3 (C-2), 137.6 (C-3), 177.0 (C-4), 162.0 (C-5), 98.5 (C-6), 164.3 (C-7), 92.7 (C-8), 157.8 (C-9), 105.4 (C-10), 123.6 (C-11), 130.7 (C-12, 16), 116.3 (C-13, 15), 160.6 (C-14) .

Elemental analysis of compound **3** was corresponded to the molecular formula $C_{15}H_{10}O_6$ that was confirmed by the presence of molecular ion peak $[M]^+$ at m/z 286 in its EI-mass spectrum . It gave olive green colour with $FeCl_3$ and positive Shinoda's test, which indicates the presence of flavonoidal skeleton (9). Its IR spectrum displayed two absorption band at 3480 and 3260 cm^{-1} for chelated and non-chelated OH groups respectively. Other IR absorption bands were observed at 1645 and 1610 cm^{-1} (α,β-unsaturated C=O) and 1510, 1415 cm^{-1} (stretching of ether function).

The ^{1}H-NMR spectrum of compound **3** displayed two *meta*-coupled doublet (*J*=2.0 Hz) each for 1H, at δ 6.13 (H-6) and 6.44 (H-8) and two *ortho*-coupled A_2B_2-type doublet (*J*=8.7 Hz) at δ 6.81 (H-13, 15) and 8.09 (H-12, 16) in aromatic region. These signals in NMR spectrum suggested the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl ring in the molecule. The presence 1,4-di-substituted phenyl was further confirmed to be *p*-hydroxyphenyl system by the ^{13}C-chemical shift of the carbon signals at δ 130.7 (C-12, 16) and 6 116.3 (C-13, 15), which fairly corresponded with those of hydrogen bearing carbons of *p*-cresol (δ 115.3, 130.2) (10).

The ^{13}C-NMR spectrum also showed the presence of C=O group at δ 177.0, a benzylic carbon (C-2) at δ 148.3 and an oxygen bonded ethylenic carbon (C-3) at δ 137.6. The downfield ^{13}C-chemical shifts of aromatic carbon atoms at δ 162.0 (C-5), 164.3 (C-7), and 160.6 (C-14) as compared to ^{13}C-chemical shifts of other aromatic carbon atoms confirmed the presence of a flavonoidal skeleton (11,12). These ^{13}C-chemical shifts suggested that C-5, C-7 and C-14 carbon atoms are substituted with phenolic functions.

On the basis of above spectral data compound **3** was identified as kaempferol. It was further confirmed by comparison of spectral data with reported data (11) and co-TLC and MMP with an authentic sample.



COMPOUND: 4

It was crystallized from MeOH as yellow needles, M.P. 226-227°C.

Elemental Analysis: Found values C=63.98%, H=3.97%, required values for $C_{16}H_{12}O_6$; C=64.00%, H=4.00%; Molecular weight 300.

IR (ν_{max}^{KBr}): cm^{-1} 3480, 3260, 1650, 1600, 1500, 1420, 1350, 1280, 1220, etc.

EI-MS: m/z 300 $[M]^+$, 285, 281, 269, 167, 153, 133, 107, 77

^{1}H-NMR (300 MHz, CD_3OD): δ 3.83 (3H, *s*, -OMe), 6.23 (1H, *d*, *J*=2.5 Hz, H-6), 6.49 (1H, *d*, *J*=2.5 Hz, H-8), 6.87 (2H, *d*, *J*=8.7 Hz, H-13, 15), and 8.06 (2H, *d*, *J*=8.7 Hz, H-12, 16)

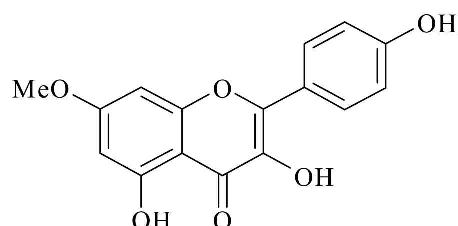
^{13}C-NMR (75 MHz, CD_3OD): δ 148.3 (C-2), 137.4 (C-3), 177.4 (C-4), 162.1 (C-5), 98.5 (C-6), 167.0 (C-7), 92.7 (C-8), 158.1 (C-9), 105.4 (C-10), 123.6 (C-11), 130.7 (C-12, 16), 116.3 (C-13, 15), 160.6 (C-14), 56.4 (-OMe) .

Compound **4** was found to have molecular weight 300 as indicated by the presence of molecular ion peak at m/z 300 in EI-positive mass spectrum. Its elemental analysis of corresponded to the molecular formula $C_{16}H_{12}O_6$. Like compound **3** it gave olive green colour with $FeCl_3$ and positive Shinoda test which indicate the presence of flavonoidal skeleton [3]. The IR spectrum of compound **4** displayed two absorption band at 3480 and 3260 cm^{-1} for chelated and non-chelated OH groups respectively. The IR spectrum also showed absorption bands at 1650 and 1600 cm^{-1} indicated presence of α,β-unsaturated C=O and at 1500, 1420 cm^{-1} for stretching of ether function.

The ^{1}H-NMR and ^{13}C-NMR data of compound **4** are similar to those of compound **3**. The ^{1}H-NMR spectrum displayed two *meta*-coupled doublet (*J*=2.0 Hz) each for 1H at δ 6.23 (H-6) and 6.49 (H-8) and two *ortho*-coupled A_2B_2-type doublet (*J*=8.7 Hz) at δ 6.87 (H-13, 15) and 8.06 (H-12, 16) in aromatic region. These proton signals suggested the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl ring. The later ring was further confirmed to be *p*-hydroxyphenyl system from the ^{13}C-chemical shift of the carbon signals at δ 130.7 (C-12, 16) and δ 116.3 (C-13,15) which fairly corresponded with those of hydrogen bearing carbons of *p*-cresol (δ 115.3, 130.2) (10).

In aliphatic region the ^{1}H-NMR spectrum displayed an integrated singlet for 3H at δ 3.83 was assigned for -OMe group attached at C-7 position, which is confirmed by downfield ^{13}C-chemical shift of C-7 at δ 167.0. The ^{13}C-NMR spectrum also showed the presence of C=O group at δ 177.4, a benzylic carbon (C-2) at δ 148.3 and oxygen bonded ethylenic carbon (C-3) at δ 137.4.

On the basis of these spectral data compound **4** was identified as rhamnocitrin; kaempferol-7-O-methyl ether. It was further confirmed by comparison of spectral data with reported values (13) and co-TLC and MMP with an authentic sample.



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