CHEMICAL EXAMINATION OF MARCHANTIA POLYMORPHA LINN.

The genus Marchantia is widely distributed both in the plains and the hills up to an altitude of 6000 meters. The thallus is identical to its name (liveworts) with broad dichotomously branched overlapping dorsiventral thallus, with goblet shaped gemmae-cup with toothed margin can easily be recognised.

Marchantia is cosmopolitan in distribution. Stephani (1900) described 67 species of the genus from different parts of the world. From India he recorded six species mostly from Himalayas, viz. M. polymorpha Linn., M. similana St. M. subintegra, Mill. M. nepalensis Lehm. et lindenbg, M. assamica Griff. and M. palmata Nees, among which M. polymorpha and M. palmata are widely distributed in the hills and the plains.

**M. polymorpha** is widely distributed e.g. Himalayas (Griffith) 1984a,b; Stephani 1900); Himalaya and Tibet, Ladakh et Nubra 3, 353 to 4267 meters; Jammu 1,828 meters; Sikkim 1,218-2438 meters, (Mitten 1860-61) common in Western Himalayas above 2,438 meters, Garhwal, Kedar Nath 3,353 meters, Pangii, Ladakh, Leh, Kargil, Kinlung, near Bara Lachapass 4,572 meters and Kashmir; (Kashyap 1929) Kotagiri, Madras, Nagapatam, Kodaikanal and Darjeeling (Chopra 1938, 1938a); Sikkim and Bengal Western Himalayas and South India (Chopra 1943), Mount Abu., Craig Wonj; (Bapna 1958; Bapna and Vyas 1962) Darjeeling, Shillong and Pynunsula 6400 meters (Singh 1966).

**M. palmata** Nees; Himalayas (Stephani 1900) Kumaon, Kashmir 2,438 meters, Lahore, Sialkot, Calcutta, Assam and South India (Kashyap 1929), Nilgiri, Hills, Kodai Kanal, Nagapatam Palni hills, Darjeeling, Sikkim and Kurseong (Chopra 1938, 1938a), Sikkim and Bengal, Western Himalayas and South India (Chopra 1943), Pandu, Kashi hills. Tejpur, Jorhat, Dibrugarh and Sibsagar (Kachroo 1952) Pachmarhi, Jambu Dwip and Chotta waterfall (Pande and Srivastava, 1952).

The plant with its dark green colour, richly dichotomous branched dorsi-ventral thallus, and more or less conspicuous midrib marked on the ventral surface in the form of ridge, and goblet shaped gemmae cup with toothed margin can easily be recognised.

In India **Marchantia polymorpha** grows both in the plains and the hills up to an altitude of 6000 meters. It occurs in humid climate in tropical subtropical and temperate zones, the plant grows near the water banks.
Male thallus

Marchantia polymorpha Linn.

Female thallus, dorsal view
The plants generally grow with mosses and both are lime lovers (John 1907). The plants start growth with early rains (June), fruiting starts in July or August. Capsules are formed in October, the plants persist throughout the winter and perishes with the outcome of summer. Occasionally some species are annuals, which persists throughout the summers along the stream or shady moist places. Sometimes they undergo a dormant period in summers and with the rains they grow again by vegetative propagation. The phenomenon may continue for years together and one may count the age of the single thallus accordingly (Fry 1928) "Like concephalum it shows its growth in winter and when it again pushes forth from the dormant apex, it elongates rapidly. Thus one is able to trace the years of growth fairly well, i.e., 3-4 years" M. polymorpha Linn.

Plants: thin large in overlapping patches margin lobed or irregular, midrib low, pores numerous, little elevated with several concentric rings, inner pore of varying shape; from quadrate to papillate, scales in six rows; Gemmae cup with lobed margin, lobed dentate. Female-head convex, raised rays normally 10, sometimes 11 bent down-ward longly truncate, margin entire, apex acute.

Plants medium: pores small with several concentric ring, inner pore quadrate, scales in four rows, midrib dark convex. Female-head flat, hemispherical disc broad, rays 7-10, short, incurved apex emarginate.
Previous Work

The studies include six orders of the liverworts namely Marchantiales, Metzgeriales, Jungermanniales, Takakiales and Anthoceratiales including 53 species. Flavonoid pattern change was observed during vegetative and reproductive stage in Marchantia berteroana. Existence of geographical races supported by the study of two different samples from U.S.A. and West Germany in Conocephalum conicum. The chemotaxonomic relevance of the flavonoid pattern in Metzgeria conjugata, M. laptoneura and the taxonomic position of the Spaerocarpus and Riella supports the view that the later two genera should come under order Marchantiales on the basis of flavonoid chemistry.

The studies on flavonoid pattern in liverworts has been worked out in the order Marchantiales, Metzgeriales, Jungermanniales, Takakiales, and Anthoceratiales, including about 53 species of the different genera. Apart from Molish (1911), flavone C-glycoside was first reported in Hepaticae and the isolation of different types of sugars from Trichocolea tomentella and flavone O-glycoside from Monoclea foresteri and Reboulia hemispherica. The occurrence of mixed O and C-glycosides, isovitexin in Porella platyphylla and the presence of flavone O-glycosiduronic acids in order Marchantiales, which includes: 7-0-rhamnosyl-galacturonide of acacetin in R. hemispherica, a derivative of 8-methoxyluteolin 7-4' di-0-galacturonide in M. foresteri; 7-0-glucuronides of apigenin, chrysoeriol and tricin in Marchantia foliacea; luteolin 3,4'-di-0-glucuronide in Lunularia cruciata. The major flavonoids of Riceia crystallina are naringenin and its 7-0-glucosides, apigenin 7-0-glucosides and apigenin 7-0-glucuronide and its derivatives; and in Ricciocarpus
natans. The major flavonoid isolated are: luteolin 7, 3'-di-0-glucuronide. Besides lucenins, five previously unknown di-C-glycosides of tricin were isolated from Plagiochila aspleniodes.

The major flavonoid of Marchantia berterroana are hypolaetin 8-0-β-D-glucuronide accompanied by apigenin and luteolin isoscetellarein (8 hydroxy apigenin) 8-0-β-D-glucuronide and galacturonide of apigenin and luteolin and the presence of major flavone glucuronides in Marchantia macropora from New Zealand. The predominance of flavone O-glucuronides is of great taxonomic interest as such glycosides are rare in the nest of the plant kingdom.

A group of workers have also established the presence of terpenes and polysaccharides in a few samples of liverworts collected in USA and West Germany.

Present Investigation:

The literature survey do not reveal a single species found in India which has been examined chemically. Therefore, a systematic chemical examination of the whole plant was undertaken. The plants were collected from Darjeeling and Assam.

Chemical examination of the whole plant of Marchantia polymorpha

The air dried plant material was powdered and extracted successively with petrol-ether and aqueous acetone since acetone extraction avoided the problem of ester formation because it has been reported that such
esters form under quite mild condition when extraction was done with methanol or ethanol.

**Constituents of the petrol ether extract**

The petrol ether extract was concentrated and chromatographed over neutral alumina using hexane, benzene, chloroform and methanol as successive eluants.

**Compound A**

The petrol ether-benzene eluates on concentration and crystallization from methanol gave shining flakes, mp. 134-135°C, (α) D+28° analysed for C_{29}H_{50}O. Mass spectrum m/e 414+ with other fragmentation peak at 399 (M-CH$_3$), 396 (M-HOH), 381 (M-CH$_2$-HOH), 329, 303, 275, 273 (M-side chain) 255 (M-side chain-HOH). The acetate prepared by pyridine and acetic anhydride in the usual manner after crystallization melted at 128°C, analysed for C$_{31}$H$_{52}$O$_2$. The compound was finally confirmed as β-sitosterol through mixed mp, CO-TLC and superimposable IR spectrum with an authentic sample (Fig. 1).

**Compound B**

The benzene eluate on concentration and crystallization with methanol gave white crystalline compound mp, 171°C (α) = 51.0° analysed for C$_{29}$H$_{48}$O. Mass spectrum m/e 412+ with other peaks at m/e 397 (M-CH$_3$) 394(M-HOH), 369,351, 300,271(M-side chain-2H),255(M-side chain-HOH). The monoacetate prepared by pyridine and acetic anhydride in the usual manner after crystallization melted
at 139° analysed for $C_{31}H_{50}O_2$. The compound was finally identified to be stigmasterol through mixed mp. and superimposable IR with an authentic sample (Fig. 2).

**Compound C**

The benzene-chloroform eluates after crystallization with methanol gave a white crystalline compound mp. 158° analysed for $C_{28}H_{48}O$. Mass spectrum m/e 400 with other peaks at m/e 385 (M-CH$_3$), 382 (M-HOH), 367 (M-CH$_3$-HOH), 315, 289, 273 (side chain) 261, 231 and 213. The acetyl derivative prepared by pyridine and acetic anhydride after crystallization melted at 138° analysed for $C_{30}H_{50}O_2$. The final identity of the compound was established as campesterol, through mixed mp. CO-TLC and superimposable IR spectrum with an authentic sample (Fig. 3).

**Constituents of the Aq. Acetone extract**

The defatted plant material was extracted with aqueous acetone. The extract was concentrated and extracted with ethyl acetate. Paper chromatography$^{22}$ of the ethyl acetate extract revealed the presence of six fluorescent spots named D, E, F, G, H, I when observed under long range UV light. Flavonoid separation$^{23}$ was carried out in Whatman No. 3 MM paper using Butanol : acetic acid : water (3:1:1); 15 percent acetic acid as solvent system. Individual compounds were isolated by means of paper chromatography using IDPC and 2 DPC techniques. Concentrate of the solvent extract were applied to several 3 M sheet and and band observed in high concentration
β-Sitosterol

Stigmasterol

Campesterol
under UV light were cut and eluted with methanol purified by paper and column chromatography.

**Compound D**

The UV spectral data of the compound in methanol showed band at 267, 296sh and 336 nm. With the addition of NaOMe a bathochromic shift in the spectrum appear on 275, 324 and 392. With the addition of NaOAc which is a weaker base than NaOMe, the bathochromic shift is less than that was in case of NaOMe (e.g. 274, 301 and 376 nm) conforming the presence of OH group in position 7. The AlCl₃/HCl spectrum of 5 OH flavone typically consists of four major absorption peaks band Ia, Ib, IIA, IIB (Fig. 4). The characteristic UV spectrum suggested the compound D to be a phenolic having phenolic hydroxy at 5, 7 and 4' position. The Rf value of the compound was found to be 0.88 (Butanol : Acetic acid : water) and 0.11 (15 percent acetic acid). The final identity of the compound was established as apigenin through mixed mp, CO-TLC and superimposable IR with an authentic sample (Fig. 5).

**Compound E**

The UV spectral data in methanol showed peaks at 242sh, 255, 268, 290sh and 348 nm. With the addition of NaOMe a bathochromic shift occurs (265sh, 328sh, 402 nm). With the addition of NaOAc, the bathochromic shift was less than that it was in case of NaOMe (e.g. 270sh, 325sh and 386sh). The characteristic UV spectrum suggested the compound to be phenolic. The Rf value in TBA 0.77 and 15 percent acetic acid 0.08. The spot appearance
U. V. SPECTRUM OF APIGENIN

\[ \text{MeOH} \]
\[ \text{MeOH} + \text{NaOMe} \]

\[ \text{MeOH} + \text{AlCl}_3 \]
\[ \text{MeOH} + \text{AlCl}_3 + \text{HCl} \]

\[ \text{MeOH} + \text{NaOAc} \]
\[ \text{MeOH} + \text{NaOAc} + \text{H}_3\text{BO}_3 \]
U.V. SPECTRUM OF LUTEOLIN

MeOH
MeOH + NaOMe

MeOH + AlCl₃
MeOH + AlCl₃ • HCl

MeOH + NaOAc
MeOH + NaOAc • H₃BO₃
in UV light was deep purple. The final identity of the compound was established as luteolin by GO-TLC and superimposable IR spectrum with an authentic sample (Fig. 6).

**Compound F**

The compound F on hydrolysis with hydrochloric acid liberated a sugar which was analysed by paper chromatography using several solvent systems e.g. ethyl acetate : pyridine : water (2:2:1); benzene : acetic acid : methanol (65 : 23.5 : 11.5) and was found to be glucuronic acid run along by the side of the concentrate as reference standard. Rf value of sugar was 0.34 in Butanol : pyridine : water (2:2:1). It was further confirmed by means of CO-TLC and superimposable IR spectrum with an authentic sample.

The aglycone had Rf 0.80 in TBA (3:1:1) 0.10 in 15% acetic acid, UV spectra bands at:

\[
\lambda_{\text{MeOH}}^{\text{max}} = 269, 334 \text{ nm.}
\]

Mass spectra m/e M+ 270.

IR spectral data exhibited carbonyl and hydroxyl absorption peaks at 1700 cm\(^{-1}\) (CO) and 3400 cm\(^{-1}\) (OH). The spectral data of compound F in different solvent system was:

\[
\lambda_{\text{MeOH}}^{\text{max}} = 369, 331 \text{ nm.}
\]

\[
\lambda_{\text{MeONa}}^{\text{max}} = 269, 287\text{sh}, 388 \text{ nm.}
\]

\[
\lambda_{\text{AcONa}}^{\text{max}} = 268, 359\text{sh}, 388 \text{ nm.}
\]
\[ \text{AlCl}_3 \text{ max} \quad 272, 296, 345, 378 \text{ nm}. \]
\[ \text{AlCl}_3/\text{HCl} \text{ max} \quad 274, 297, 354, 378 \text{ nm}. \]

The above spectral data suggested the aglycone to be apigenin and that C-7 in compound F is substituted with glucuronic acid. Therefore, the identity of the compound was established as apigenin 7-0-glucuronide (Fig. 7).

**Compound G**

The compound G on hydrolysis with hydrochloric acid liberated a sugar which was analysed by paper chromatography using several solvent systems e.g. ethyl acetate : pyridine : water (2:2:1); benzene : acetic acid : methanol (65:23.5:11.5) and was found to be glucuronic acid run along by the side of the concentrate as reference standard. Rf values of sugar was 0.34 in Butanol : pyridine : water (2:2:1). It was further confirmed by means of CO-TLC and superimposable IR spectra with an authentic sample.

The aglycone had Rf 0.68 in TBA (3:1:1) 0.05 in 15% acetic acid : UV spectra bands at MeOH 256, 365sh, 343 nm.
Mass spectra m/e M⁺ 286.
IR spectra data exhibited carbonyl and hydroxyl absorption peaks at 1700 cm⁻¹ (CO) and 3400 cm⁻¹ (OH). The spectral data of compound G in different solvent system was:
\[ \text{MeOH max} \quad 253, 266sh, 345 \text{ nm}. \]
\[ \text{MeONa max} \quad 259, 398 \text{ nm}. \]
\[ \text{AcONa max} \quad 256, 264sh, 406 \text{ nm}. \]
U. V. SPECTRUM OF COMPOUND - H

MeOH • AlCl₃
MeOH • AlCl₃ • HCl

λ nm

200 300 400 500
The above spectral data suggested the aglycone to be luteolin and that C-7 in compound G is substituted with glucuronic acid. Therefore, the identity of the compound was established as luteolin 7-O-glucuronide (Fig. 7).

**Compound H**

The UV spectral data for the compound H was found to be:

\[ \text{AcONa/H}_3\text{BO}_3 \max \ 257, 263\text{sh}, 370 \text{nm.} \]
\[ \text{AlCl}_3 \max \ 273, 290\text{sh}, 331, 427 \text{nm.} \]
\[ \text{AlCl}_3/\text{HCl} \max \ 268, 290 \text{sh.} \]

The UV spectral data suggested that the compound possess free hydroxyl group at 5 and 7 but lack orthodihydroxyl groups in the B ring. The free 5
The hydroxyl group is evident by the shift observed with AlCl₃/HCl and the free 7 hydroxyl group by the bathochromic shift of band II with NaOAc. It is thus clear that compound H* is a 3', 4'-diglycosylated luteolin. This glycosylation pattern must account for the unusual UV spectrum of compound H which with band I absorption at 316 nm. is more typical of 5,7 dihydroxy-flavone than of a luteolin derivative. Hypsochromic shifts in band I of flavone spectra have been observed previously when 5-7-3'- or 4' glycosylation is present but the extent of these shifts is usually of the order of only 3-10 nm. in monoglycosides and 14-16 nm in 7, 4'-diglycosides. In the present case 3'-4'-diglycosylation has produced a hypsochromic shift of almost 30 nm. The sugar moiety was suspected to be a uronic acid since it was resistant to acid hydrolysis. This was confirmed by hydrolysis with β-glucuronidase and pectinase (polypecturonidase). β-glucuronidase was found to convert compound H to luteolin where as pectinase treatment for the same time period gave only small quantities of luteolin. Luteolin was identified by chromatography, UV spectral analysis and CO-TLC and superimposable IR with an authentic sample. The sugars produced after hydrolysis were analysed by paper chromatography. The glucuronic acid (as distinct from galacturonic acid) was produced.

The stereochemistry of the sugar aglycone linkage is defined by the β-glucuronidase hydrolysis and thus the compound could be luteolin 3', 4'-di-O-β-D-glucuronide. The compound appeared to be a new product isolated from the Indian species of the plant material for the first time (Fig. 8).
### Compound I

The UV spectral data of the compound was as follows:

\[
\text{MeOH} \quad \lambda_{\text{max}} \quad 240\text{sh}, 268 \text{ and } 336 \text{ nm.}
\]

\[
\text{NaOMe} \quad \lambda_{\text{max}} \quad 257, 267\text{sh}, 391 \text{ nm.}
\]

\[
\text{NaOAc} \quad \lambda_{\text{max}} \quad 257, 264\text{sh}, 292\text{sh}, 393 \text{ nm.}
\]

\[
\text{NaOAc/H}_3\text{BO}_3 \quad \lambda_{\text{max}} \quad 267, 340 \text{ nm.}
\]

\[
\text{AlCl}_3 \quad \lambda_{\text{max}} \quad 275, 292\text{sh}, 344, 379 \text{ nm.}
\]

\[
\text{AlCl}_3/\text{HCl} \quad \lambda_{\text{max}} \quad 275, 292\text{sh}, 344, 379 \text{ nm.}
\]

Spot colour in UV light + NH_3 \quad \text{Yellow}

Rf. value TBA \quad 0.18

15 percent acetic acid \quad 0.19

Partial conversion of the compound I by acid hydrolysis gives a product X. The compound X on hydrolysis with 6 percent hydrochloric acid at 100° liberated a sugar which was analysed by paper chromatography using different solvent system.

The liberated sugar was run along with authentic glucuronic acid as reference standard on paper in different solvent system like ethyl acetate: pyridine : water (2:2:1); Benzene : acetic acid : methanol (65:23:5:11.5). It was further confirmed by means of CO-TLC and CO-IR comparison with an authentic sample. The Rf value of the aglycone in TBA (3:1:1) was 0.70 and in 15 percent acetic 0.05. UV spectra \[
\text{MeOH} \quad \lambda_{\text{max}} \quad 256, 365\text{sh}, 343 \text{ nm and}
\]

Mass spectra m/e 286 M^+ was identical with luteolin. IR spectral data was
U.V. SPECTRUM OF COMPOUND-1

\[ \text{MeOH} \cdot \text{AlCl}_3 \]

\[ \text{MeOH} \cdot \text{AlCl}_3 \cdot \text{HCl} \]
Apigenin

Luteolin

Apigenin-7-0-glucuronide

Luteolin-7-O-glucuronide

Luteolin 3', 4'-di-O-β-D-glucuronide

Luteolin 7, 4'-Di-O-β-D-glucuronide
3400 (OH) and 170 (C = O). The conversion of X to luteolin and glucuronic acid by means of acid hydrolysis indicated the compound X to be 7-glucuronide of luteolin.

The β-glucuronidase hydrolysis of the compound I gives the compound Y. The UV spectral data of the compound Y in Methanol and its bathochromic shift by the addition of NaOMe and also by estimation of the Rf value of the compound Y in different solvent system suggested the compound to be luteolin. From the spectral data of compound I (in AlCl₃/HCl shift) it is seen that it possess a free 5-hydroxyl group. In NaOMe the compound I does not exhibit a 58 nm bathochromic shift of band I with an increase in intensity which is indicative that 4'-hydroxyl group is not free. Thus, the compound I does not contain a free 4'-hydroxyl group and so must be a 4'-glycosylated luteolin. This data defines the glycosation pattern of compound I. The mobility of I on paper is in the region expected for luteolin diglycoside. Compound I must be the diglycoside, luteolin 7,4'-di-0-β-D-glucuronide (Fig. 9). This is the first record of the compound in an Indian species of Marchantia. The above type of flavonoid components identified in Marchantia polymorpha has not been reported from any liverwort found in India and this is the first report from Indian species of Marchantia.

**Experimental**

The melting point (uncorrected) were determined by a Gallenkamp melting point apparatus, unless stated otherwise. IR spectrum were recorded in KBr, Alumina and Silica gel (BDH) were activated at 120° before use in the chromatographic column. The solvents used for chromatography were
moisture free. Thin layer chromatography were carried out on silica gel G. (S. Merck).

Chemical examination of Marchantia polymorpha

The air dried whole plant collected from Assam and Darjeeling was coarsely powdered and extracted with petrol ether and aqueous acetone respectively.

Constituents of the petrolether extract

The petrol ether extract after concentration was chromatographed over neutral alumina using hexane benzene, chloroform and methanol as successive eluants.

Compound A- β- sitosterol

The petrol ether-benzene eluates on concentration gave a Lieberman-Burchardt positive compound crystallized from methanol mp. 134-135° (α) D= +28°.

Analysed for found C, 84.12; H, 12.16%
Calcd. C, 84.05; H, 12.07%

IR peaks at 3450 cm\(^{-1}\) (OH).

Acetate of β-sitosterol

β-sitosterol in pyridine and acetic anhydride was left for 20 hours at room temperature. After the usual processing of the reaction mixture, it afforded a monoacetate derivative crystallized from methanol, mp. 128° (α) D= + 28°.
Compound B-stigmasterol

The benzene eluate on concentration and crystallization from methanol gave white crystalline compound mp. 171°C; (α) D = -51°C.

Analysis found C, 84.32; H, 11.52%
C_{29}H_{48}O

Analysis calculated C, 84.47; H, 11.65%

Mass spectrum: m/e 412 (M^+), 397 (M-CH_3), 394 (M-HOH), 369, 351, 300, 271 (M- side chain - 2H).

Acetyl derivative of stigmasterol

Stigmasterol in pyridine and acetic anhydride was kept overnight and the reaction mixture after working up in the usual manner gave white mono-acetyl derivative crystallized from methanol mp. 139°C.

Analysis found C, 81.86; H, 11.08%
C_{31}H_{50}O_2

Analysis calculated C, 81.94; H, 11.01%

Compound C- Campesterol

The benzene-chloroform eluates after concentration and crystallization from methanol, gave a white crystalline compound mp. 158°C.

Analysis for found C, 84.12; H, 12.06%
C_{28}H_{48}O

Analysis calculated C, 84.00; H, 12.00%

Mass spectrum: m/e 400 (M^+), 385 (M-CH_3), 382 (M-HOH), 367 (M-CH_3HOH), 315, 289, 273 (side chain) 261, 231 and 213.
Acetyl derivative of campesterol

Campesterol in pyridine and acetic anhydride was kept overnight and the reaction mixture after working up in the usual manner gave a white compound, crystallized from methanol mp. 138°.

Analyzed

\[ \text{C}_{30}\text{H}_{50}\text{O}_{2} \]

Calcd.

\[ \text{C}, 81.45; \text{H}, 11.31\% \]

Constituents of the aqueous acetone extract

The defatted plant material was extracted with aqueous acetone for 24 hours. Acetone extract was concentrated under reduced pressure and reextracted with ethyl acetate. The ethyl acetate extract after concentration gave shinoda positive residue. All flavonoids separation were carried out on Whatman 3 MM paper using T-BuOH/Acetic acid/water (3:1:1) and 15 percent acetic acid for 2 DPC. TBA, HOAc solvent system were found to be most satisfactory for the 2 DPC analysis of the flavonoids. The ethyl acetate concentrate on chromatographic examination revealed the presence of five ammonia sensitive fluorescent spots when observed under long range UV light.

Separation of flavonoids

Flavonoid separation were carried out in Whatman No. 3 MM paper using tert. Butanol : acetic acid : water (3:1:1) and 15 percent acetic acid solvent system. Individual compound were isolated by IDPC and 2 DPC technique. Concentration of solvent extracts were applied to several 3 MM Whatman chromatographic paper and bands observed in high concentration under
UV light were cut out, eluted with methanol, purified by means of paper and column chromatography. Six compound D, E, F, G, H, I were isolated.

**Compound D- Apigenin**

**UV-spectral data**

| MeOH $\lambda_{max}$ | 267, 296sh, 336 nm. |
| NaOMe $\lambda_{max}$ | 275, 324, 392 nm. |
| NaOAc $\lambda_{max}$ | 274, 301, 376 nm. |
| AlCl$_3$/HCl $\lambda_{max}$ | 276, 299, 340, 381 nm. |

**Rf value**

- Butanol : acetic acid : water (4:1:5) 0.88
- 15 percent acetic acid 0.11

**Spot appearance**

- UV light Deep purple
- UV/NH$_3$ Yellow green

**Compound E- Luteolin**

| MeOH $\lambda_{max}$ | 242sh, 255, 268, 290sh, 348 nm. |
| NaOMe $\lambda_{max}$ | 265sh, 328sh, 402 nm. |
| NaOAc $\lambda_{max}$ | 270, 325, 386 nm. |
Rf value

Butanol : acetic acid : water (4:1:5) 15 percent acetic acid

0.77 0.08

Spot appearance

UV light Deep purple
UV/NH₃ Yellow

Compound F-Apigenin 7-O-glucuronide

The compound was hydrolysed with 6 percent hydrochloric acid at 100° for 2 hours. The aglycone was extracted with ether and the sugar was analysed by paper chromatography and thin layer chromatography using different solvent system like ethyl acetate : pyridine : water (2:2:1), benzene acetic acid : Methanol (65:23.5:11.5). The sugar was identified to be glucuronic acid when a reference standard of glucuronic acid was run along the unknown sugar.

Rf value of the sugar-Butanol : pyridine : water (2:2:1) - 0.34

Study of the aglycone:

The aglycone was identified as apigenin by chromatographic and ultraviolet spectrum analysis:

Rf value

Butanol : acetic acid : water (3:1:1) 15 percent acetic acid

0.80 0.10
**UV spectra**

\[
\text{MeOH} \quad \lambda_{\text{max}} = 269, 334 \text{ nm.}
\]

**Mass spectrum**  
- \(m/e \ M^+ = 270\)

**IR spectral data**

Absorption peaks at 3400 cm\(^{-1}\) (OH), 1700 cm\(^{-1}\) (CO).

**UV spectral data of compound F**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>(\lambda_{\text{max}}) (nm)</th>
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<tbody>
<tr>
<td>MeOH</td>
<td>269, 331</td>
<td>269, 331</td>
</tr>
<tr>
<td>NaOMe</td>
<td>269, 287sh, 388</td>
<td>269, 287sh, 388</td>
</tr>
<tr>
<td>NaOAc</td>
<td>268, 259sh, 388</td>
<td>268, 259sh, 388</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>272, 296, 345, 378</td>
<td>272, 296, 345, 378</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>274, 297, 354, 378</td>
<td>274, 297, 354, 378</td>
</tr>
</tbody>
</table>

**Compound G-luteolin 7-O-glucuronide**

The compound G on hydrolysis with hydrochloric acid liberated a sugar which was analysed by paper chromatography using several solvent systems e.g., ethyl acetate : pyridine : water (2:2:1), benzene : acetic acid : Methanol (65:23.5:11.5) and was found to be glucuronic acid run along by the side chain of the concentrate as reference standard of Rf values of sugar was 0.34 in Butanol : pyridine : water (2:2:1). It was further confirmed by means of CO-TLC and superimposable IR spectra with an authentic sample.
Study of the aglycone

The aglycone was identified as luteolin by chromatographic and ultraviolet spectrum analysis:

Rf value
TBA (3:1:1) 0.68
15% acetic acid 0.05

UV spectra
\[
\begin{align*}
\text{MeOH} & \quad \lambda_{\text{max}} \quad 256, 365 \text{ sh}, 343 \text{ nm}. \\
\text{MeONa} & \quad \lambda_{\text{max}} \quad 259, 398 \text{ nm}. \\
\text{AcONa} & \quad \lambda_{\text{max}} \quad 256, 264 \text{ sh}, 406 \text{ nm}. \\
\text{AcONA/H}_3\text{BO}_3 & \quad \lambda_{\text{max}} \quad 257, 263 \text{ sh}, 370 \text{ nm}. \\
\text{AlCl}_3 & \quad \lambda_{\text{max}} \quad 273, 290 \text{ sh}, 331, 427 \text{ nm}. \\
\text{AlCl}_3/\text{HCl} & \quad \lambda_{\text{max}} \quad 268, 290 \text{ sh}. 
\end{align*}
\]

Mass spectrum  m/e M⁺ 286

IR spectral data
Absorption peaks at 3400 cm⁻¹ (OH), 1700 cm⁻¹ (CO).

UV spectral data of compound G

\[
\begin{align*}
\text{MeOH} & \quad \lambda_{\text{max}} \quad 253, 266 \text{ sh}, 345 \text{ nm}. \\
\text{MeONa} & \quad \lambda_{\text{max}} \quad 259, 398 \text{ nm}. \\
\text{AcONa} & \quad \lambda_{\text{max}} \quad 256, 264 \text{ sh}, 406 \text{ nm}. \\
\text{AcONA/H}_3\text{BO}_3 & \quad \lambda_{\text{max}} \quad 257, 263 \text{ sh}, 370 \text{ nm}. \\
\text{AlCl}_3 & \quad \lambda_{\text{max}} \quad 273, 290 \text{ sh}, 331, 427 \text{ nm}. \\
\text{AlCl}_3/\text{HCl} & \quad \lambda_{\text{max}} \quad 268, 290 \text{ sh}. 
\end{align*}
\]

Compound H⁺ - luteolin 3', 4'-di-O-β-D-glucononide

UV spectral data
\[
\begin{align*}
\text{MeOH} & \quad \lambda_{\text{max}} \quad 267, 316 \text{ nm}. 
\end{align*}
\]
\[
\begin{align*}
\lambda_{\text{max}} \text{ (NaOMe)} & : 272, 300 \text{ sh, } 341 \text{ nm.} \\
\lambda_{\text{max}} \text{ (NaOAc)} & : 272, 303 \text{ sh, } 340 \text{ sh.} \\
\lambda_{\text{max}} \text{ (NaOAc/H}_3\text{BO}_3 \text{)} & : 267, 315 \text{ nm.} \\
\lambda_{\text{max}} \text{ (AlCl}_3 \text{)} & : 278, 289 \text{ sh, } 328, 378 \text{ sh.} \\
\lambda_{\text{max}} \text{ (AlCl}_3/\text{HCl) } & : 278, 289 \text{ sh, } 328, 378 \text{ sh.}
\end{align*}
\]

Spot colour in UV light: Dark

Rf value:
- TBA: 0.27
- 15% acetic acid: 0.42

Hydrolysis of the compound with $\beta$-D-glucuronidase

The glycoside was dissolved in pH 5 buffer (an aqueous 0.5 M NaOAc solution adjusted to pH 5 with HOAc) and powdered enzyme $\beta$-D-glucuronidase added. The mixture was allowed to stand overnight at 37°. When the solution was concentrated under high vacuum and paper chromatographed only luteolin was detected.

UV data of luteolin

\[
\begin{align*}
\lambda_{\text{max}} \text{ (MeOH)} & : 240 \text{ sh, } 252, 267, 290 \text{ sh, } 348 \text{ nm.} \\
\lambda_{\text{max}} \text{ (NaOMe)} & : 266 \text{ sh, } 328 \text{ sh, } 401 \text{ nm.} \\
\lambda_{\text{max}} \text{ (NaOAc)} & : 268 \text{ sh, } 325 \text{ sh, } 384 \text{ nm.}
\end{align*}
\]
Rf value:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>TBA</td>
<td>0.77</td>
</tr>
<tr>
<td>15% acetic acid</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Spot appearance in UV light: Deep purple.

Compound I* Luteolin 7, 4'-di-O-β-D-glucuronide

UV spectral data

<p>| | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>MeOH max</td>
<td>240 sh, 268, 336 nm.</td>
<td></td>
</tr>
<tr>
<td>NaOAc max</td>
<td>257, 267 sh, 391 nm.</td>
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</tr>
<tr>
<td>NaOAc/H3BO3 max</td>
<td>257, 264 sh, 292 sh, 393 nm.</td>
<td></td>
</tr>
<tr>
<td>AlCl3 max</td>
<td>275, 292 sh, 344, 379 nm.</td>
<td></td>
</tr>
<tr>
<td>AlCl3/HCl max</td>
<td>275, 292 sh, 344, 379 nm.</td>
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</tr>
</tbody>
</table>

Spot colour in UV/NH3: Yellow.

Rf value:

<p>| | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>TBA</td>
<td>0.18</td>
</tr>
<tr>
<td>15% acetic acid</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Partial hydrolysis of compound I

Compound I was hydrolysed with hydrochloric acid for one hour on a steam bath where by a compound X was obtained which on chromatographic and UV spectral analysis showed that the compound X was luteolin 7-glucuronide. The compound I on hydrolysis with B-glucuronidase as described earlier gave luteolin as the aglycone and glucuronide as the sugar identified by Rf and UV spectral analysis (as described earlier for luteolin and glucuronic acid). Sugar was identified a glucuronide by chromatographic method.

References

5. S.R. Kashyap, Liverworts of Western Himalayas and the Punjab Plains, 1, Lahore.


