

Extraction, Isolation and Identification

Air dried bran (1 kg) was finely powdered and exhaustively extracted with petroleum ether (40–60°C) to remove fats, lipids and resinous materials. Then, it was subjected to fractional extraction using acetone followed by ethyl alcohol. The acetone and ethanolic extracts were subjected to two-dimensional paper chromatography using the solvent systems [BuOH : AcOH : H₂O (4 : 1 : 5)] and AcOH (15%) respectively, which revealed the presence of many components of phenolic and flavonoid nature. The preparative paper chromatography (ppc) using Whatman 3 mm papers and the solvent systems (4 : 1 : 5) or AcOH (15%) for irrigation, was used to isolate the compounds of the two extracts. These compounds were further purified over Sephadex LH-20 column prior to physical and chemical analyses. Complete and controlled acid hydrolyses (2N HCl, 1 h and 0.1 N HCl, resp.) of the glycosides under investigation yielded the sugar residues and the aglycones. All of which were co-chromatographed with authentic samples. Enzymic hydrolysis⁸ was achieved using β -glucosidase or β -galactosidase. All UV data were recorded in MeOH and in the presence of diagnostic reagents.⁸ The ¹H-NMR spectra of the trimethylsilyl ethers of all flavonoids were recorded in CDCl₃ at 90 MHz and reported at δ -values (ppm) relative to TMS as an internal standard on a Bruker WM 90 apparatus.

The antibacterial effect of the extracts was studied by using the agar-diffusion method⁹ on six different strains of bacteria *i.e.*, two gram-negative namely *Escherichia coli* and *Brodetella brochiseptica* and four gram-positive namely *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus pumilus*, and *Bacillus subtilis*. Bacterial test organisms were cultured on nutrient agar slant media¹⁰ and incubated at 37°C for 24 h. The antimicrobial assay has been carried out by cup-plate diffusion technique.⁹ The clear zone of inhibition around the cup was measured in millimetres (diameter of cup 10 mm). Ten mg of each extract were dissolved in 2 mL diethyl sulfoxide and 8 mL distilled water to obtain 10 mL solvent; 0.1 mL of each tested against the above test organisms. The plates were incubated at 37°C for 24 h. The antibacterial activity was measured as growth zone of inhibition of microorganisms. All the tests were run in triplet for each sample and the means of inhibition zones were given to assess the activity.

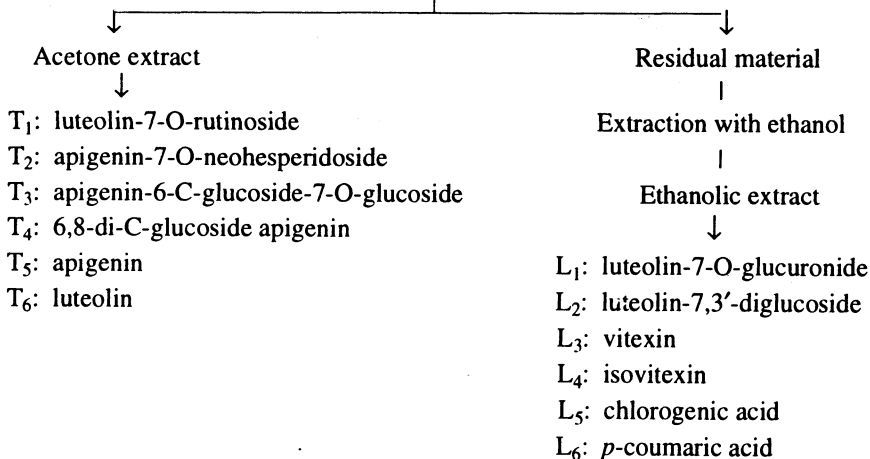
RESULTS AND DISCUSSION

This study deals with the investigation of the polyphenolic constituents of *Triticum aestivum* (wheat bran, Sakha 69). After removal of resinous and fatty material using petroleum ether (40–60°C), the wheat bran was extracted with acetone followed by ethyl alcohol as shown in the scheme. The acetone extract was subjected to (ppc) on Whatman 3 mm sheets irrigated in the solvent system 15 % AcOH, whereby 2 flavone glycosides, 2 C-glycosides and 2 aglycones were isolated and identified as: luteolin-7-O-rutinoside, apigenin-7-O-neohesperidoside, 6,8-di-C-glucoside apigenin, 6-C-glucoside-7-O- β -D-glucoside apigenin, apigenin and luteolin. While luteolin-7-O-glucuronide, -7,3'-O- β -D-diglucoside, 8-C-glucoside apigenin (vitexin), 6-C-glucoside apigenin (isovitexin), chlorogenic and *p*-coumaric acids were isolated from the ethanolic extract by (ppc) using

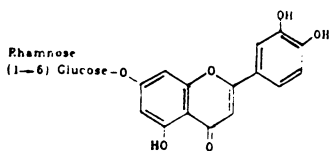
(4 : 1 : 5) for irrigation. The structure of the isolated compounds was identified through R_f -values and colour reactions (Tables 1 and 3), UV spectral data in MeOH and in the presence of diagnostic reagents (Tables 2 and 4), complete and controlled acid hydrolyses, enzymic hydrolysis and $^1\text{H-NMR}$ spectroscopy (data are recorded in Table-5).

Triticum aestivum (Wheat bran, Sakha 69)

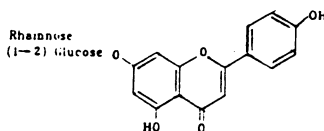
Extraction with petroleum ether (40–60°C)
followed by acetone



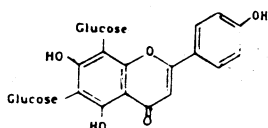
Compounds Isolated from Wheat Bran



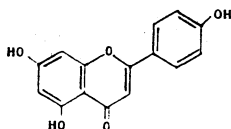
T₁: Luteolin-7-rutinoside



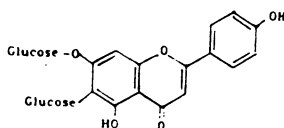
T₂: Apigenin-7-neohesperidoside



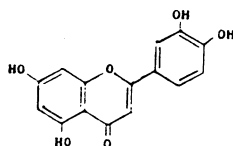
T₃: 6,8-di-C-glucoside apigenin



T₄: Apigenin



T₅: Apigenin-6-C-glucoside-7-O-glucoside



T₆: Luteoli

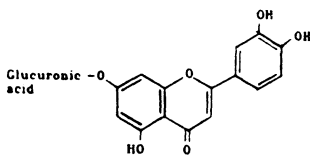
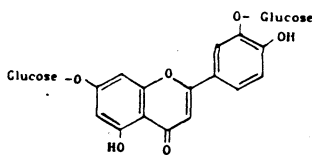
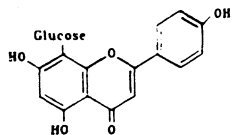
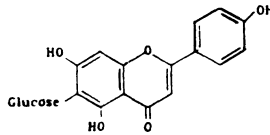
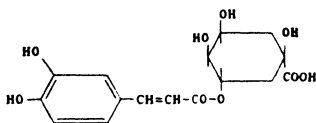
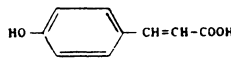
L₁: Luteolin-7-O-glucuronideL₂: Luteolin-7-3'-diglucosideL₃: VitexinL₄: IsovitexinL₅: Chlorogenic acidL₆: *p*-Coumaric acid

TABLE-1
R_F-VALUES AND COLOUR REACTIONS OF THE COMPOUNDS
ISOLATED FROM ACTONE EXTRACT

| | R _F -values × 100 | | | | Colour reactions | | | | | | |
|----------------|------------------------------|----------|------------------|--------|------------------|------------|--------------|--------------|-------------------|-------------------|--------------|
| | | | | | Untreated | | Ammonia | | FeCl ₃ | AlCl ₃ | |
| | BAW | 15% AcOH | H ₂ O | Phenol | Visible | UV | Visible | UV | Visible | Visible | UV |
| T ₁ | 37 | 30 | 5 | 60 | — | Brown | Faint yellow | Yellow | Green | Faint yellow | Yellow |
| T ₂ | 38 | 36 | 5 | — | — | Brown | Faint yellow | Yellow | Green | Faint yellow | Yellow |
| T ₃ | 31 | 50 | 14 | 41 | — | Purple | — | Faint Yellow | — | — | Yellow green |
| T ₄ | 87 | 57 | — | 89 | — | Purple | — | Yellow | Green | — | Yellow |
| T ₅ | 42 | 64 | 31 | 65 | — | Purple | — | Faint yellow | — | — | Yellow green |
| T ₆ | 78 | 66 | 8 | 66 | — | Dark brown | — | Yellow | Green | — | Yellow |

T₁ = Luteolin-7-rutinosideT₃ = Apigenin-6,8-di-C-glucosideT₅ = Apigenin-6-C-glucoside-7-glucosideT₂ = Apigenin-7-neohesperidose.T₄ = ApigeninT₆ = Luteolin

TABLE-2
UV-SPECTRAL DATA (λ_{\max} nm) OF THE COMPOUNDS ISOLATED
FROM ACETONE EXTRACT

| | MeOH (a) | (a) + NaOMe (b) | (a) + AlCl ₃ (c) | (c) + HCl (d) | (a) + NaOA (e) | (e) + H ₃ BO ₃ (f) |
|----------------|-------------------------|-------------------------|--------------------------------|------------------------|------------------------|---|
| T ₁ | 274, 311*, 333 | 383, 331*, 398 | 281, 306, 352, 390 | 280, 304*, 346, 386 | 308, 334*, 383, 395 | 276, 284*, 323*, 351 |
| T ₂ | 255, 265*, 349 | 263, 299*, 394 | 272, 296*, 331, 432 | 272, 295, 359, 389 | 259, 266*, 366, 403 | 258, 370 |
| T ₃ | 254, 265, 349 | 263, 405 | 272, 295, 402 | 272, 294, 360*, 392 | 256, 264, 352, 403 | 258, 263*, 359 |
| T ₄ | 271, 336 | 249*, 271, 304*, 389 | 268*, 277, 301, 352, 381 | 279, 300, 344, 378 | 261*, 271, 350, 392 | 269, 341 |
| T ₅ | 241*, 252, 290*, 347 | 265*, 330*, 402 | 275, 301*, 326, 425 | 276, 295*, 352, 386 | 268, 325*, 385 | 258, 300*, 372, 425* |
| T ₆ | 267, 296*, 336 | 275, 324, 392 | 276, 301, 348, 384 | 276, 299, 340, 381 | 274, 301, 376 | 268, 301*, 338 |

*Shoulder

TABLE-4
UV-SPECTRAL DATA (λ_{\max} nm) OF THE COMPOUNDS ISOLATED
FROM ETHANOL EXTRACT

| | MeOH (a) | (a) + NaOMe (b) | (a) + AlCl ₃ (c) | (c) + HCl (d) | (a) + NaOA (e) | (e) + H ₃ BO ₃ (f) |
|----------------|-------------------|--------------------|--------------------------------|--------------------------------|------------------------|---|
| L ₁ | 255, 264*, 353 | 264, 300*, 407 | 274, 302*, 357, 373 | 267*, 274, 365, 370 | 255, 262*, 364, 404 | 259, 377 |
| L ₂ | 255*, 269, 337 | 279, 394 | 274, 302*, 352, 387* | 275, 300*, 344, 384 | 270, 280*, 309, 352 | 269, 345 |
| L ₃ | 270, 302*, 336 | 279, 329, 395 | 277, 305, 350, 386 | 278, 303, 343, 383 | 280, 300, 379 | 271, 329, 344 |
| L ₄ | 271, 336 | 278, 329, 398 | 262*, 278, 304, 352, 382 | 260*, 280, 302, 344, 380 | 279, 303, 385 | 274, 346, 408* |
| L ₅ | 245, 330 | — | — | — | — | — |
| L ₆ | 227, 310 | 335 | — | — | — | — |

*Shoulder

TABLE-3
R_f-VALUES AND COLOUR REACTIONS OF THE COMPOUNDS ISOLATED FROM ETHANOL EXTRACT

| | R _f -values × 100 | | | | | | Colour reactions | | | | | | | | |
|----------------|------------------------------|--------------|------------------|--------|-----|---------|------------------|---------|--------------|---------------------|-------------------|--------------|-------------------|---------|----|
| | BAW | 15 % AcOH | H ₂ O | Phenol | BAW | | Untreated | | Ammonia | | FeCl ₃ | | AlCl ₃ | | |
| | | | | | | Visible | UV | Visible | UV | Visible | UV | Visible | UV | Visible | UV |
| L ₁ | 24 | 25 | 12 | 17 | 14 | — | Dull brown | — | Faint yellow | Bright yellow green | Green | Faint yellow | Yellow | — | — |
| L ₂ | 30 | 27 | 12 | 55 | — | — | Dark | — | — | Light yellow | Green | — | Yellow | — | — |
| L ₃ | 43 | 24 | 7 | 72 | 38 | — | Deep purple | — | — | Yellow green | Green | — | Yellow green | — | — |
| L ₄ | 62 | 46 | 19 | 89 | 42 | — | Deep purple | — | — | Yellow green | Green | — | Yellow green | — | — |
| L ₅ | 63 | 62 | 68 | — | — | — | Blue | — | — | Green | Green | — | — | — | — |
| L ₆ | 92 | — | 42 | — | — | — | Blue | — | Violet | Green | Green | — | — | — | — |

L₁ = Luteolin-7-galactoside, L₂ = Luteolin-7-3-diglucoside, L₃ = Vitexin, L₄ = Isovitexin, L₅ = Chlorogenic acid, L₆ = *p*-coumaric acid.

TABLE-5: ¹H-NMR DATA OF SOME OF THE ISOLATED COMPOUNDS

| Compounds | Aglycone moiety δ (ppm) | Sugar moiety δ (ppm) |
|-------------------------------|--|--|
| Luteolin-7-O-rutinoside | 7.41 (dd, J = 2.5 Hz and J = 9 Hz, H-6'); 7.3 (d, J = 2 Hz, H-2'); 6.9 (d, J = 8 Hz, H-5'); 6.43 (s, H-3); 6.4 (d, J = 2.5 Hz, H-6). | 5.0 (d, J = 7.5 Hz, H-1'' of glucose); 4.3 (d, J = 2 Hz, H-1''' of rhamnose); 0.86 (d, J = 6.5 Hz, CH ₃ of rhamnose); 3.53–3.9 (m of rutinose protons). |
| Apigenin-7-O-neohesperidoside | 7.78 (d, J = 9 Hz, H-6' and H-2'); 6.88 (d, J = 8.5 Hz, H-5' and H-3'); 6.5 (d, J = 2 Hz, H-8); 6.35 (s, H-3); 6.2 (d, J = 2 Hz, H-6). | 5.2 (d, J = 7.5 Hz, H-1'' of glucose); 4.98 (d, J = 2 Hz, H-1''' of rhamnose); 1.28 (d, J = 6 Hz, CH ₃ of rhamnose); 3.5–3.9 (m of neohesperidose protons). |
| 6, 8-di-C-glucoside apigenin | 7.86 (d, J = 9 Hz, H-2' and H-6'); 7.04 (d, J = 9 Hz, H-3' and H-5'); 6.4 (s, H-3). | 4.7 (d, J = 7.5 Hz, H-1'' of glucose); 4.5 (d, J = 7.5 Hz, H-1''' of glucose); 3.0–4.2 (m of 12 sugar protons). |
| Apigenin | 7.8 (d, J = 9 Hz, H-6' and H-2'); 6.9 (d, J = 8.5 Hz, H-5' and H-3'); 6.55 (d, J = 2 Hz, H-8); 6.3 (s, H-3); 6.22 (d, J = 2 Hz, H-6). | |
| Luteolin | 7.44 (dd, J = 2.5 Hz and J = 9 Hz, H-6'); 7.35 (d, J = 2 Hz, H-2'); 6.55 (s, H-3); 6.44 (d, J = 2.5 Hz, H-8); 6.3 (d, J = 2.5 Hz, H-6). | |
| Luteolin-7-O-glucuronide | 7.4 (dd, J = 2.5 Hz, and J = 9 Hz, H-6'); 7.3 (d, J = 2 Hz, H-2'); 6.92 (d, J = 8 Hz, H-5'); 6.7 (d, J = 2.5 Hz, H-8); 6.44 (s, H-3); 6.38 (d, J = 2.5 Hz, H-6). | 4.9 (d, J = 7.5 Hz, H-1''); 3.2–3.8 (m of glucuronide protons). |
| 8-C-β-D-glucoside apigenin | 8.0 (d, J = 8 Hz, H-6' and H-2'); 6.98 (d, J = 8 Hz, H-5' and H-3'); 6.27 (s, H-6); 6.78 (s, H-3). | 4.72 (d, J = 7.5 Hz, H-1''); 3.3–3.88 (m of glucose protons). |
| 6-C-β-D-glucoside apigenin | 8.0 (d, J = 8 Hz, H-6' and H-2'); 6.89 (d, J = 8 Hz, H-5' and H-3'); 6.3 (s, H-8); 6.78 (s, H-3). | 4.75 (d, J = 7.5 Hz, H-1''); 3.2–3.8 (m of glucose protons). |

s = singlet, d = doublet, dd = double doublet, m = multiplet, J = coupling constant.

The study of the antibacterial effect of the two extracts showed that the acetone extract had a slight effect on two of the gram-positive bacteria only from the six strains while the ethanolic extract had also a light effect on one of the gram-negative bacteria and one of the gram-positive bacteria as shown in the following Table:

| Extracts | Antibacterial activity | | | | | |
|-------------------|------------------------|-----|------------------------|------|--------|--------|
| | Gram-negative bacteria | | Gram-positidve bactria | | | |
| | Es. | Br. | St. | S.a. | Ba. p. | Ba. s. |
| Acetone extract | - | - | - | + | - | + |
| Ethanolic extract | + | - | - | - | - | + |

Bacteria: Es. = *Escherichia coli*, Br. = *Brodetella brochiseptica*, St. = *Staphylococcus aureus*, S.a. = *Sarcina lutea*, Ba. p. = *Bacillus pumilus*, Ba. s. = *Bacillus subtilis*

Zone of inhibition (mm): (+) from 11–13 mm

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(Received: 21 July 1997; Accepted: 20 October 1997)

AJC-1375