Some Polyphenolic Constituents of *Triticum aestivum* (Wheat bran, Sakha 69) and Their Antibacterial Effect

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The polyphenolic constituents of *Triticum aestivum* (wheat bran, Sakha 69) (Gramineae) were investigated through extraction first with acetone and then with ethyl alcohol. The acetone extract showed to contain 2 flavone glycosides, 2 C-glycosides and 2 aglycones, which were identified as luteolin-7-O-rutinoside, apigenin-7-O-neohesperidoside, 6,8-di-C-glucoside apigenin, apigenin-6-C-glucoside-7-O-glusoside, apigenin and luteolin. The ethanolic extract proved to contain luteolin-7-glucuronide, 7,3'-diglucoside, vitexin, isovitexin, chlorogenic and *p*-coumaric acids. All the isolated and purified components were thoroughly identified using both physical and chemical methods of analyses and were further confirmed by the spectral measurements. The antibacterial effect of the two extracts was studied by using the agar-diffusion technique on six different strains of bacteria.

INTRODUCTION

Triticum aestivum (wheat) is one of the most important plants of the family Gramineae due to its valuable econmic importance. The C-glycosylflavones found in wheat leaves¹ were a varied mixture of compounds in the luteolin and apigenin series; lutonarin, lucenin-l and -3, vicenin-2, wyomin, isoswertisin-4′-O-glucoside and -7-O-glucoside. Tricin, tricin-5-glucoside, -5-diglucoside and 6-C-(rhamnosylglucosyl)-luteolin were isolated from Triticum dicoccum.² While vicenin-1-sinaposyl and isoschaftoside sinapoyl were found in Triticum wheat germ.³ More than 30 flavone-C-glycosides were present in leaves of diploid Triticum species.⁴ Feng et al.⁵ identified 2 C-glycoside flavones from bran of red spring wheat. Due to the antimicrobial activity of many of the phenolic and flavonoid compounds against different bacterial and fungal strains,6,7 the antibacterial effect of the acetone and ethanol extracts of Triticum aestivum (wheat bran, Sakha 69) were studied on six different microorganisms (bacteria).

EXPERIMENTAL

Plant material

Triticum aestivum (wheat bran, Sakha 69) was obtained from the Agriculture Research Centre, Giza, Egypt..

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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 \(\beta\)-glucosidase or \(\beta\)galactosidase. All UV data were recorded in MeOH and in the presence of diagnostic reagents. 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 media 10 and incubated at 37°C for 24 h. The antimicrobial assay has been carried out by cup-plate diffusion technique. 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.

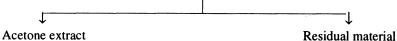
RESULTS AND DISCUSSION

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 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



T₁: luteolin-7-O-rutinoside

T₂: apigenin-7-O-neohesperidoside

T₃: apigenin-6-C-glucoside-7-O-glucoside

T₄: 6,8-di-C-glucoside apigenin

T₅: apigenin

T₆: luteolin

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Extraction with ethanol

Ethanolic extract

L₁: luteolin-7-O-glucuronide

 L_2 : luteolin-7,3'-diglucoside

L₃: vitexin L₄: isovitexin

L₅: chlorogenic acid L₆: p-coumaric acid

Compounds Isolated from Wheat Bran

T₁: Luteolin-7-rutinoside

T₃: 6,8-di-C-glucoside apigenin

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

T₂: Apigenin-7-neohesperidoside

T₄: Apigenin

T₆: Luteoli

L₁: Luteolin-7-O-glucuronide

L₂: Luteolin-7-3'-diglucoside

L₃: Vitexin

L₄: Isovitexin

L5: Chlorogenic acid

L₆: p-Coumaric acid

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

			1	100			(Colour reac	tions		
	Kf	-vaiue	es × l	100	Untreated		Am	Ammonia		AlCl ₃	
	BAW	15 % ACoH	H ₂ O	Phenol	Visible	ΛΛ	Visible	ΛΩ	Visible	Visible	Δ
Tı	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	<u> </u>	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

 $T_1 = Luteolin-7$ -rutinoside

 T_3 = Apigenin-6,8-di-C-glucoside

 T_5 = Apigenin-6-C-glucoside-7-glucoside

 T_2 = Apigenin-7-neohesperiodose.

 $T_4 = Apigenin$

 $T_6 = Luteolin$

	MeOH (a)	(a) + NaOMe (b)	(a) + AlCl ₃ (c)	(c) + HCl (d)	(a) + NaOA (e)	(e) $+ H_3BO_3$ (f)
Tı	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_3BO_3 (f)
Lı	255, 264*, 353	264, 300*, 407	274, 302*, 357, 373	267*, 274, 365, 370	255, 262*, 364, 404	259, 377
I_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

Rr-VALUES AND COLOUR REACTIONS OF THE COMPOUNDS ISOLATED FROM ETHANOL EXTRACT

		ď	Re-values × 100	9				Ŭ	Colour reactions	18		
						Untre	Untreated	Ammonia	onia	FeCl ₃	AICI3	.l ₃
	BAW	15 % AcOH	H ₂ O	Phenol	BAW	Visible	nv u	Visible	UV	Visible	Visible	NN
L ₁ 24 25	24	25	12	17	41		Dull	Faint	Bright yellow green	Green	Faint	Yellow
L_2	30	27	12	55	1 .	1	Dark	1	Light yellow	Green	1	Yellow
L ₃	43	24	7	. 22	38	1	Deep purple	1	Yellow green	Green	I	Yellow green
7	62	46	16	68	42	I	Deep	1	Yellow	Green		Yellow green
Ls	63	62	89	1	١	-	Blue	ł	Green	Green	1	1
L ₆ 92	92	1	42	1	1		Blue	l	Violet	Green	l	١

 L_1 = Luteolin-7-galactoside, L_2 = Luteolin-7-3-diglucoside, L_3 = Vitexin, L_4 = Isovitexin, L_5 = Chlorogenic acid, L_6 = 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-neohespe- ridoside	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-glucuro- nide	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:

			Antibacter	tibacterial activity			
Extracts	Gram-nega	tive bacteria	Gram-posidtive 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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