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A new acylated luteolin glycoside from *Curcuma Longa* L. and free radical scavenging potential of its extracts

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The ethanol and water extracts of turmeric (*Curcuma longa* L.) displayed free radical scavenging activity. Chemical investigation of the ethanolic extract led to the isolation of a new acylated luteolin glucoside, luteolin-7-O-(6"-p-hydroxybenzoyl-β-D-glucopyranoside) (compound 1), together with the known flavonoids: luteolin 7-O-β-D-glucopyranoside (compound 2), apigenin-7-O-β-D-glucopyranoside (compound 3), luteolin (compound 4) and apigenin (compound 5) which were isolated for the first time from *C. longa* L. rhizomes, in addition to three diphenylheptanoids: curcumin (compound 6), demethoxycurcumin (compound 7) and bisdemethoxycurcumin (compound 8). The diphenylheptanoids have been previously reported for *C. longa.*

Key words: *Curcuma longa* L., polyphenols constituents, luteolin-7-O-(6"-p-hydroxybenzoyl-β-glucopyranoside).

INTRODUCTION

The rhizomes of turmeric (*C. longa* L., Zingiberaceae) play an important role as a remedy for stomach and liver ailments. Medicinal uses of the rhizomes arise from their contents of volatile oil and diphenylheptanoids (curcuminoids) (Pothitirat and Gritsanapan, 2006). Turmeric has been reported to possess anti-inflammatory, hepatoprotective, antiviral activities, and anticancer activity (Radha et al., 2006; Fryer et al., 2009) through its effects on gene expression (Aggarwal et al., 2003). Curcuminoids exhibited free radical scavenging property (Ramsewak et al., 2002), antiproliferative and immunomodulatory activities (Grace et al., 2010). *Curcuma longa* L. extracts protected against cardiotoxicity induced by doxorubicin (El-Sayed et al., 2011).

Turmeric oil is composed of several monoterpene and sesquiterpene compounds such as zingiberene and α- and β-turmerone. It is used as carminative, antifungal and as antiplatelet agent (Lee, 2006). In Ayurvedic system of medicine, the rhizomes of *C. longa* were used as a stimulant, tonic, stomachic and depurative (Challopadhyay et al., 2004). *C. longa* is referred by different names in different culture.

This study reported the isolation and identification of a new compound from the ethanol extract of the dried powder of *C. longa* L. rhizomes: luteolin-7-O-(6"-p-hydroxybenzoyl-β-D-glucopyranoside) (compound 1) together with seven known phenolic compounds 2 to 8. Free radical-scavenging property of both the ethanol and water extracts were tested by means of electron spin resonance (ESR) assay using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Acqua and Innocenti, 2004) and vitamin C as positive control.

EXPERIMENTAL

Plant

Rhizomes of *C. longa* L. were purchased from a herbal shop at Cairo on November 2009 and were identified by Prof. I. El Garf, Faculty of Science, Cairo university. A voucher specimen has been

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deposited in the herbarium of the National Research Centre, Cairo, Egypt.

Preparation of the plant extracts for antioxidant activity

The ground-dried plant was separately extracted with 80% ethanol and water by percolation until exhaustion, the extracts were filtered and the solvents were evaporated under reduced pressure at low temperature until dryness.

Isolation and identification

The finely powdered rhizomes of *C. longa* L. (500 g) was extracted with 80% ethanol (EtOH 3×3 L) at 80°C. The combined extracts were evaporated under reduced pressure at 45°C to give 25 g of dark brown residue. The ethanol extract was fractionated on a polyamide S6 CC and eluted with water followed by gradient increasing ethanol proportions with decreasing polarity, obtaining four fractions (I to IV).

Fraction I (10% EtOH) was found to be free from phenolic compounds. Fraction II (20% EtOH) was chromatographed on Silica gel 60 G column chromatography (CC) using EtOAC and EtOAC-MeOH increasing gradient polarity up to pure MeOH, to afford compounds 6 (41 mg), 7 (20 mg) and 8 (33 mg). Fraction III (40% EtOH) was chromatographed by preparative paper chromatography (PPC) using Whatman No. 3 MM paper with (S1) n-BuOH-HOAc-H_2O (4:1:5, top layer), (S2) 15% aqueous HOAc and Sephadex LH-20 CC, eluted with methanol to give yellow powder of compounds 1 (15 mg), 2 (40 mg) and 3 (23 mg). Fraction IV (50% EtOH) was subjected to CC on Sephadex LH-20 with 50% aqueous MeOH as an eluent to give compounds 4 (50 mg) and 5 (30 mg). All separation processes were followed up by 2D-PC and CoPC using Whatman 1 paper with (S1) and (S2) as solvent systems.

Luteolin-7-O-(6''-p-hydroxybenzoyl-β-glucopyranoside) (compound 1):

Amorphous powder: R_f in (S1) 0.45. R_f in (S2): 0.32, exhibited a dark purple color in UV light. UV λmax (MeOH) nm: 256, 271, 349, (NaOMe): 265, 299sh, 395, ($AlCl_3$): 273, 299sh, 329, 430 ($AlCl_3$/HCl): 273, 299sh, 356, 387 (NaOAc): 265, 360, 403, (NaOAc/H_3BO_3): 264, 375. ^{1}HNMR and ^{13}CNMR (300 and 125 MHz, DMSO-d_6) (Table 1).

Evaluation of antioxidant activity of ethanol and water extracts using DPPH as stable free radical

This was carried out by electron spin resonance measurement according to the described method (Acqua and Innocenti, 2004; Makhmoor, 2003). The ability of each extract to scavenge the free radical DPPH was measured. 10 mg of ethanol extract and water extracts were dissolved in 1 ml methanol. DPPH (Sigma, Aldrich) was also prepared at concentration of 10 mg/1 ml methanol (as a source of stable free radical). The standard solution was prepared from vitamin C (Cid company) at concentration of 10 mg/ml methanol and functioned as antioxidant.10 µl of each of the test solutions and the standard were added to190 µl of DPPH solution. The negative control was prepared from 0.2 ml DPPH. All test and standard solutions were incubated at 37°C for 30 min. Electron spin resonance measurement of DPPH was recorded and the percentage inhibition of the free radical was calculated from the double integration areas (DIA). It had previously been verified that addition of methanol did not affect the DPPH signal.

$$\text{Inhibition (\%)} = \frac{\text{DIA (DPPH)} - \text{DIA (DPPH + extract)}}{\text{DIA (DPPH)}} \times 100$$

RESULTS AND DISCUSSION

The ethanol extract was chromatographed on polyamide column S6 followed by successive separation on preparative paper chromatography (PC), silica gel column chromatography(CC) and sephadex LH-20 (CC) to afford pure samples of a new acylated luteolin glucoside: luteolin-7-*O*-(6''-p-hydroxybenzoyl-*β*-D-glucopyranoside) (compound 1) together with luteolin-7-*O*-*β*-D-glucopyranoside (compound 2), apigenin-7-*O*-*β*-D-glucopyranoside (compound 3), luteolin (compound 4), apigenin (compound 5) and three diphenylheptanoids: curcumin (compound 6), demethoxycurcumin (compound 7) and bisdemethoxycurcumin (compound 8). Their structures (Figure 1) were established on the basis of chemical evidences, their chromatographic properties and spectroscopic study (UV, ^{1}HNMR, ^{13}CNMR and ESI-MS). Compound 1 was expected to be acylated luteolin 7-*O*-glycoside on the basis of its chromatographic properties (R_f values, dark under UV light, change with ammonia vapors and $FeCl_3$ reagent). UV spectrum in methanol gave characteristic band I at *λ*max 349 nm and band II at 256 nm of a luteolin nucleus (Mabry et al., 1970) together with a maximum at 271 nm to indicate the acylation of the glycoside moiety with a phenoyl group. No bathochromic shift in band II after addition of NaOAc reagent indicated the glycosylation of 7-OH and the increase in intensity of band I on addition of NaOMe suggested a free 4'-OH group.

Other UV spectra after the addition of the different diagnostic shift reagents were in a good agreement with those reported for 7-O-glycosyl luteolin (Mabry et al., 1970).

On complete acid hydrolysis (2N aqueous HCl, 3 h, 100°C), compound 1 gave D-glucose in the aqueous phase as has been confirmed by comparative paper chromatography, while p-hydroxybenzoic acid and luteolin were detected in the organic phase (CoPC). Accordingly, it was tentatively identified as luteolin-7-*O*-(p-hydroxybenzoyl-*β*-D-glucoside). This evidence was confirmed from its negative ESI-MS analysis. The molecular ion peak observed in its negative ESI-MS at m/z 567 $[M-H]^-$ (corresponding to MF $C_{28}H_{24}O_{13}$) together with diagnostic fragment ion peak at 447 [M-p-hydroxybenzoyl]$^-$, 285 [luteolin-H]$^-$ confirm the earlier supposed structure.

^{1}H-NMR spectrum of compound 1 showed an ABX coupling system at *δ* ppm 7.50dd, 7.43d and 6.85d, assigned to H-6', H -2' and H-5' for 3',4'-dihydroxy B-ring and an AX system of two *meta* doublets at 6.78 and 6.44 assigned to H-8 and H-6, respectively. An AX coupling system of two *ortho* doublets at 7.60 and 6.67 for H-2'''/6''' and H-3'''/5''', indicated the presence of a p-hydroxybenzoyl acyl part on the sugar moiety. *δ*- and *J*-values of the anomeric proton signal (5.01 ppm and 7.5 Hz) were diagnostic evidences for *β*-configuration and $^4C_1$ conformation of the glucoside moiety. Chemical shifts

Table 1. NMR spectral data (300 and 75 MHz, DMSO-d_6) of compound 1.

H/C	^{13}CNMR (δppm)	^{1}HNMR (Δppm)
Luteolin		
1	-	-
2	164.47	-
3	103.10	6.70(s)
4	181.83	-
5	161.10	-
6	99.50	6.44 (d, J=2 Hz)
7	162.92	-
8	94.72	6.78 (d, J=2 Hz)
9	156.90	-
10	105.30	-
1'	121.30	-
2'	113.57	7.43 (d, J=2 Hz)
3'	145.79	-
4'	149.95	-
5'	116.03	6.85(d, J=8.5 Hz)
6'	119.06	7.50(dd, J=8.5 and 2 Hz)
Glucose		
1''	99.96	5.01 (d, J=7.5 Hz)
2''	73.12	-
3''	76.39	-
4''	69.57	-
5''	77.20	-
6''	64.27	4.24 (dd, J=12.5 and 4.8 Hz,H-6''$_a$); 4.12 (dd, J=12.5 and 2.5 Hz,H-6''$_b$)
p-hydroxy-benzoate		
1'''	120.97	-
2'''/6'''	133.12	7.60 (d,J=8.5 Hz)
3'''/5'''	116.60	6.67 (d,J=8.5 Hz)
4'''	163.47	-
7'''	167.60	-

of carbon resonances of glucose agreed with the reported data (Agrawal, 1992).

Location of the benzoyl moiety at C-6'' of the glucose has been deduced from the downfield shift of the two doublet of doublets of H-6$_a$'' and H-6$_b$'' at 4.24 and 4.12, respectively. The attachment of the benzoyl residue to C-6'' of glucose was further deduced from the downfield shift of C-6'' of glucose (δ 64.27 in the ^{13}C-NMR spectrum of compound 1 relative to the corresponding resonance at δ 60.55 for luteolin-7-O- β-glucoside) (Harborne and Mabry, 1982; Agrawal, 1989). This evidence was finally confirmed from the assignment of the characteristic five carbon resonances of the benzoyl moiety at 167.60, 163.47, 133.12, 120.97, 116.60 for C-7''', C-4''', C-2'''/6''', C-1''' and C-3'''/5''', respectively. The assignment of all other ^{13}C resonances was followed from its comparison with related published data (Harborne and Mabry, 1982; Agrawal, 1989). Based on these results, compound 1 was identified as luteolin-7-*O*-(6''-p-hydroxybenzoyl-β-D-glucopyranoside).

Compounds (2 to 8) were identified by comparing their chromatographic behavior, chemical and spectral data (UV, ^{1}HNMR and EI-MS) to those published: luteolin-7-O-glucoside (compound 2), apigenin-7-O-glucoside (compound 3), luteolin and apigenin (compounds 4 and 5) (Mabry et al., 1970), curcumin, demethoxycurcumin, and bisdemethoxycurcumin (compounds 6, 7 and 8) (Pill-Hoon, 2000).

The antioxidant activities of ethanol and water extracts were estimated by electron spin resonance (ESR) through inhibiting the stable free radical of DPPH, compared to vitamin C (positive control). The results were obtained by recording the double integration areas of DPPH free radical by ESR, calculated after the addition of the inhibitor (extract). The ethanol extract showed an activity as free radical scavenger at 92.3% inhibition

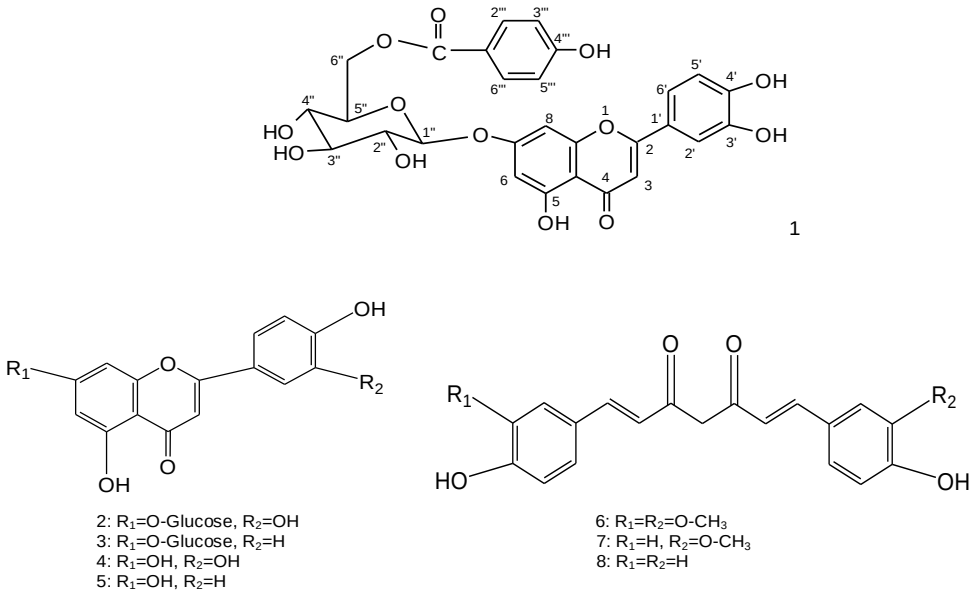

Figure 1. Chemical structures of the phenolic constituents isolated from *Curcuma longa*.

Table 2. Inhibition of DPPH radical by *Curcuma longa* extracts.

Compound	Double integration area	Inhibition (%)
DPPH	639	-
Vitamin C	0	100
Ethanol extract	29	92.3
Water extract	42.6	90.2

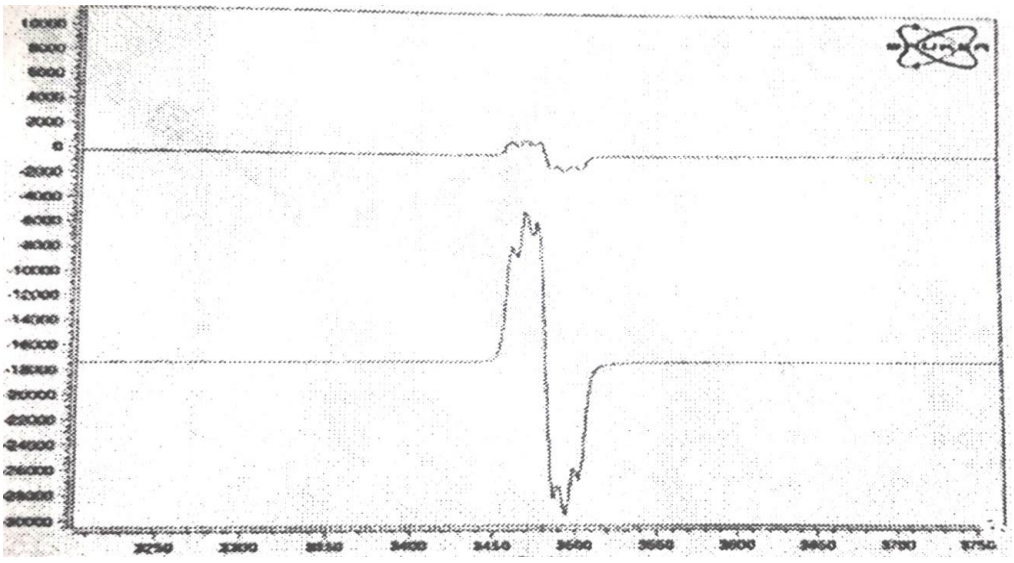

Figure 2. ESR of control DPPH and ethanol extr.of *Curcuma longa*.

(Table 2 and Figure 2) followed by water extract at 90.2% inhibition compared to vitamin C showing 100% inhibition. The antioxidant activities of *C. longa* extracts might be due to the presence of phenolic compounds such as flavonoids and curcuminoids. Flavonoids and phenolic acids are active defensive molecules in the prevention of

different pathological disorders in organisms as they are natural antioxidants (Larson, 1999). They are commonly used in industry for the prevention of oxidative degradation of polymers and natural pigments. The flavonoids have free radical scavenging activity, because of their ability to chelate the transition metal involved in the production of reactive oxygen species via the Fenton reaction (Hibatallah et al., 1999).

REFERENCES

Acqua SD, Innocenti G (2004). Antioxidant compounds from *Chaerophyllum hirsutum* extracts. Fitoterapia 75:592-595.

Aggarwal BB, Kumar A, Bharti AC (2003). Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res. 23:363-398.

Agrawal PK (1989). Studies in organic chemistry 39, ^{13}C NMR of flavonoids in Agrawal P K, Bansal M C eds, chapter 6, flavonoid glycosides , New York Elsevier science pp. 283-364.

Agrawal PK (1992). NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31(10):3307-3330.

Challopadhyay I, Biswas K, Bandyopadhyah U, Banerjee R, Ranajit K (2004). Turmeric and curcumin: Biological actions and medicinal applications. Curr. Sci. 87(1):44-53.

El-Sayed EM, Abd El-azeem AS, Afify AA, Shabana MH, Ahmed HH (2011). Cardioprotective effects of *Curcuma longa* L. extracts against doxorubicin-induced cardiotoxicity in rats. J. Med. Plants Res. 5(17):4049-4058.

Fryer RA, Galustian C, Dalgelish AG (2009). Recent advances and developments in treatment strategies against pancreatic cancer.Curr. Clin. Pharmacol. 4(2):102-112.

Grace GL, Yue BCL, Chan Po-Ming Hon, Mavis YHL, Kwok-Pui F, Ping-Chung L, Lau CBS (2010). Evaluation of in vitro anti-proliferative and immunomodulatory activities of compounds isolated from *Curcuma longa*. Food Chem. Toxicol. 48(8-9):2011-2020.

Harborne JB, Mabry TJ (1982). The Flavonoids: Advances in Research. In: Markham K.R., Mohanchari, eds, chapter 2, Carbon 13 NMR spectroscopy of flavonoids. Chapman and Hall Ltd, Cambridge, London, University press pp. 119-132.

Hibatallah J, Carduner C, Poelman MC (1999). *In-vivo* and *in-vitro* assessment of the free radical scavenger activity of *Ginkgo* flavone glycosides at high concentration. J. Pharm. Pharmacol. 51:1435-1440.

Larson R (1999). The antioxidants of higher plants. Phytochemistry 27:969-978.

Lee HS (2006). Antiplatelet property of *Curcuma longa* L. rhizome derived ar-turmerone. Bioresour. Technol. 97:1372-1376.

Mabry TJ, Markham KR, Thomas MB (1970). The Systematic Identification of Flavonoids, part II, chapter V "The Ultraviolet spectra of flavones and flavonols" Berlin, Springer-Verlag 41-164.

Makhmoor T (2003). Evaluation of antioxidant property of different extracts of *Withania somnifera*. International workshop on the development of medicines from plants H.E.J.COMSTECH (CPC)-TWAS 69-71.

Pill-Hoon B (2000). Spectral and photophysical behaviors of curcumin and curcuminoids. Bull. Korean Chem. Soc. 21(1):81-86.

Pothitirat W, Gritsanapan W (2006). Variation of bioactive components in *Curcuma longa* in Thailand. Current Sci. 91(10):1397-1400.

Radha KM, Anoop KS, Jaya G, Rikhab GS (2006). Multiple biological activities of curcumin: A short review. Life Sci. 78:2081-2087.

Ramsewak RS, De Witt DL, Nair MG (2002). Cytotoxicity, antioxidant and anti-inflammatory activites of curcumins I-III from Curcuma longa. Phytomedicine 27:303-308.