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New Flavone Glycoside from *Wisteria sinensis.*

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ABSTRACT

The chromatographic separation of extracts of *Wisteria sinensis* (family Fabaceae) gave a new flavone, 7-O-[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]-5,6,7,4'-tetrahydroxy flavone, in addition to the identification of many known compounds from the volatile fractions by the GC/MS analysis. The cytotoxicity of *W. sinensis* extracts increased as follows: petroleum ether extract against the cell line HePG2 < methylene chloride extract against HCT-116 < petroleum ether extract against MCF-7 (IC50: 23.5, 22.7, 16.7µg, respectively). Compared to ascorbic acid as a standard, the antioxidant activity of extracts was found to be weak. As antimicrobial, the butanol fraction was the strongest against fungi (*Aspergillus fumigatus* 12.6 ± 0.63, *Geotricum candidum* 13.7 ± 0.72), gram +ve bacteria *(Streptococcus pneumoniea* 15.8 ± 0.72, *Bacillis subtilis* 17.3 ± 0.58) and gram –ve bacteria (*Escherichia coli* 11.4 ± 1.2), compared to standards.

Keywords: Wisteria sinensis, Flavone glycoside, deoxy mannose, GC/MS, cytotoxicity, antioxidant, antimicrobial.

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INTRODUCTION

Wisteria is a genus of flowering plants belonging to the pea family (Fabaceae), that includes ten species of woody climbing vines native to the eastern United States and the East Asian countries of China, Korea, and Japan. It is also an extremely popular ornamental plant in China and Japan. *Wisteria sinensis* (Sims.) DC. (Chinese wisteria), is a climbing plant that can reach a height of 20 m. It is considered an invasive species in certain areas [1]. Its pendent racemes, with their many high blue-violet scented flowers, make a striking picture. The flowers of *W. sinensis* are also cured in sugar then mixed with flour and made into a famous local delicacy called "Teng Lo". The leaves and flowers are also used as a tea substitute [2]. In addition the fiber from its stems can be used to make paper [3]. *Wisteria* species are used as a food source by the larvae of some *Lepidoptera* species of moth including the brown-tail [4]. Interestingly, many oriental medicinists use *Wisteria* gall extracts for treating gastric cancer [5] and cancer of breast and stomach, or rheumatoid arthritis patients [6-13]. Several *Wisteria* species have been also reported to have antioxidant [14] and antibacterial activities [15]. A survey of the literature showed that in previous studies phenylpropanoids and *β*-chromenes have been isolated from the oil of *W. sinensis* flowers [16] and several *Wisteria* species have been found to contain triterpene saponins, [6-8], isoflavones [10-13], and lectins [17-19].

We reported here our findings concerning the isolation of a new flavone glycoside, as well as the phytochemical studies on extracts of *W. sinensis* aerial parts and the anticancer, antioxidant, antimicrobial activities of the investigated extracts.

RESULTS AND DISCUSSION

Phytochemical evaluation

Compound **1** was separated from the butanol fraction. ^{1}H NMR spectrum of **1** (Table 1) showed AA'BB' signal system at δ 6.94 and 7.90 ppm indicating 4'-oxygenated B-ring, two one-proton singlets at δ 6.68 and 6.48 ppm which could be assigned to H-3 and H-8, respectively [20], and signals of two sugar moieties with the two anomeric protons at δ 5.23 (axial H with J= 7.2) and 5.30 (equatorial H as broad singlet). The spectrum is nearly similar to that of 7-O-[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]-5,7,4'-trihydroxy flavone [21], with H-6 signal no longer present and consequently H-8 signal became singlet. Thus, the structure of **1** was proposed as 7-O-[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]-5,6,7,4'-tetrahydroxy flavone, which was confirmed by the MS spectrum that gave $[M-H]^{+}$ ion at m/z 593 with relative abundance of 7.54%. The IR spectrum gave absorption bands at 3424 (due to stretching OH groups), 2925, 2855 (due to stretching CH, CH_2, CH_3), 1657 (due to stretching aromatic C=O), 1630, 1601 (due to stretching C=C, Ph groups), 1439, 1408, 1100, 672 and 467 cm^{-1}.

The GC/MS analysis of essential oil petrolatum ether extract (Ws1) gave 18 compounds

(2-19), essential oil methylene chloride extract (Ws2) gave 20 compounds, **(6-9, 11-19, 20-26)**, and hexane extract (Ws3) gave 23 compounds, **(9-12, 15, 21, 23, 27-42)** (Table 2).

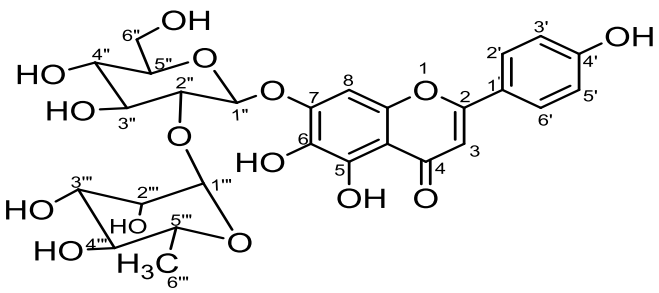

Figure 1: Chemical structure of compound 1

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Table 1: ^{1}H NMR data of compound 1

^{1}H atom	δ value, ppm	multiplicity (J, Hz)
3	6.68	s, 1H
8	6.48	s, 1H
3',5'	6.94	d, 2H, (8.8)
2',6'	7.90	d, 2H, (8.4)
1"	5.23	d, 1H, (7.2)
1"'	5.30	br s, 1H
Sugar protons	3.42-3.96	m, 10H
Sugar-CH_3	1.34	d, 3H, (6.4)

Biological applications

Cytotoxicity assessment

Table 3 indicated the in vitro cytotoxicity IC50 (µg/ml). The cytotoxicity against HePG2 of petroleum ether extract (Ws1) and methylene chloride extract (Ws4) were "moderate", butanol extract (Ws6) was "weak", against MCF-7 of petroleum ether extract (Ws1) was "strong", methylene chloride extract (Ws4) and butanol extract (Ws6) were "moderate", and against HCT-116 of petroleum ether extract (Ws1), methylene chloride extract (Ws4) and butanol extract (Ws6) were "moderate".

Table 3: Cytotoxicity assessment of *W. sinensis* extracts against human tumor cell lines HePG2, MCF-7and HCT-116

Compounds	In vitro cytotoxicity IC50 (µg/ml)•		
	HePG2	MCF-7	HCT-116
5-fluorouracil	6.6±0.24	4.7±0.11	8.4±0.20
petroleum ether (Ws1)	23.5	16.7	32.8
methylene chloride (Ws4)	41.7	20.1	22.7
butanol (Ws6)	63.5	46.3	36.2

IC50 (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic).

Free radical scavenging activity

The free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used for detection of the antioxidant activity of plant extracts [22]. The lower the IC50, the higher the antioxidant scavenging activity. Methylene chloride extract (Ws4) has the highest scavenging activity. The scavenging effect of the extracts and standard on the DPPH radical decreased in the following order: ascorbic acid, methylene chloride extract (Ws4) and ethyl acetate extract (Ws5) (Table 4).

Table 4: Antioxidant activity of the extracts of *W. sinensis* by DPPH method

Fraction	DPPH radical scavenging activity (IC_{50} µg/ml)
ascorbic acid	14.2
ethyle acetate	82.1
methylene chloride	77.8
butanol	-ve
methylene chloride (essential oil)	-ve
petroleum ether	-ve
Hexane	-ve

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Antimicrobial activity assessment

The antimicrobial potentials of butanol extract (*Ws*6), ethyl acetate extract(*Ws*5), methlyene chloride extract essential oil (*Ws*2), petroleum ether extract essential oil (*Ws*1) , hexane extract (*Ws*3), methylene chloride extract (*Ws*4) of *W. sinensis* were examined by the disc diffusion assay method, using eight pathogenic microbial species; *Aspergillus fumigates, Syncephalastrum racemosum, Geotricum candidum, Candida albicans,* represent pathogenic fungal species. *Streptococcus pneumonia, Bacillis subtillis,* represent Gram positive species, *Pseudomonas aeruginosa, Escherichia coli,* represent Gram negative species.

The data were presented in (Table 5). The results showed that there were remarkable inhibitions of the bacterial growth against the tested organisms. The inhibitions zone of hexane extract *(Ws*3) against *Aspergillus fumigates*, ethyl acetate extract (*Ws*5) and methylene chloride extract (*Ws*4) against *Geotricum candidum* were comparable to *Amphotericin B*. The inhibition zone against *Streptococcus pneumonia* and *Bacillis subtillis* of the hexane extract (*Ws*3), methylene chloride extract (*Ws* 4) and ethyl acetate extract (*Ws*5) were comparable to *Ampicillin*. The inhibition zones of the hexane extract (*Ws*3), methylene chloride extract (*Ws*4) and ethyl acetate extract (*Ws*5) against *Escherichia coli* were comparable to *Gentamicin.*

Table 5: The inhibition zone in mm of extracts of *W. sinensis* compared to standard antibiotics.

Sample / Test Microorganisms	*Ws*1	*Ws*2	*Ws*3	*Ws*4	*Ws*5	*Ws*6	Standard of antibiotic
Fungi							*Amphotericin B*
Aspergillus fumigates (RCMB 02568)	NA	13.2 ± o.72	20.1 ± 0.63	16.9 ± 0.58	16.3 ± 2.1	12.6 ± 0.63	23.7 ± 0.1
Syncephalastrum racemosum (RCMB 05922)	NA	NA	NA	NA	NA	NA	19.7 ± 0.2
Geotricum candidum (RCMB 05097)	NA	15.1 ± 0.38	21.4 ± 0.63	20.1 ± 0.58	19.6 ± 0.63	13.7 ± 0.72	28.7 ± 0.2
Candida albicans (RCMB 05036)	NA	NA	NA	NA	NA	NA	25.4 ± 0.1
Gram positive Bacteria							*Ampicillin*
Streptococcus pneumoniae(RCMB 010010)	NA	17.1 ± 0.63	20.8 ± 0.44	19.1 ± 0.63	18.6 ± 1.2	15.8 ± 0.72	23.8 ± 0.2
Bacillis subtillis (RCMB 010067)	NA	18.3 ± 1.2	21.6 ± 0.58	20.6 ± 0.63	20.3 ± 0.58	17.3 ± 0.58	32.4 ± 0.2
Gram negative Bacteria							*Gentamicin*
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	NA	NA	NA	17.3 ± 0.1
Escherichia coli (RCMB 010052)	NA	12.4 ± 0.58	21.4 ± 0.58	20.6 ± 0.58	20.3 ± 0.72	11.4 ± 1.2	19.9 ± 0. 3

*Ws*6= butanol extract; *Ws*5=ethyl acetate extract; *Ws*2= methlyene chloride extract essential oil; *Ws*1=petroleum ether extract essential oil; *Ws*3=hexane extract; *Ws*4= methylene chloride extract; NA = No test

EXPERIMENTAL

Instrumentations: ^{1}H NMR spectra were measured on Bruker Avance III 400 MHz for 1H and 100 MHz for 13C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet. Data were analyzed using Topspin 3.1 Software. IR The infrared spectrum was recorded on Mattson 5000 FT-IR spectrometer at Faculty of Pharmacy, Mansoura University. The GC/MS analysis was performed at National Research Center, Dokki, Cairo, using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251 mm, 0.1 mm film thickness).For GC/MS detection, an electron

ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 150°C (hold 4 min) to 280°C as a final temperature at an increasing rate of 5°C /min (hold 4 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Material and reagents: PTLC were performed on silica gel (Kieselgel 60, GF 254) of 0.25 mm thickness; petroleum ether (60-80), diethyl ether, hexane, methylene chloride, ethyl acetate, acetone, butanol and methanol were obtained from Adwic Company; The cell lines HePG-2, hepatocellular carcinoma (liver) and MCF-7, mammary gland (breast) were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt; The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

The cell line of heptacelluar carcinoma cell line (HepG2) was obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

The reagents RPMI-1640 medium,MTT , DMSO and 5-fluorouracil were obtained from Sigma co., St. Louis, USA), and Fetal Bovine serum from GIBCO, UK .

Plant material: *W. sinensis* was collected in April 2014 from El-Orman Botanical Garden, Giza, Egypt. The plant was authenticated by Prof. Wafaa M. Amer, Department of Botany, Faculty of Science, Cairo University, Giza, Egypt.

Processing of the plant material: Fresh plant aerial parts of *W. sinensis* (500g) were percolated in petroleum ether then subjected to hydro-steam distillation and the volatile oil was extracted by petroleum ether followed by methylene chloride to give petroleum ether extract (3.212 g) and methylene chloride extract (0.148 g). The remainder plant material, after distillation, was soaked in methanol for 48 hrs, and filtrated. The aqueous extract was extracted by hexane, followed by methylene chloride, ethyle acetate, then butanol to give the corresponding extracts (0.498, 1.558, 0.522, 2.351 g, respectively). The butanol fraction (1.50 g) gave by CC (silica ge, ethyl acetate/methanol, 13:7) followed by TLC (silica gel , ethyl acetate/H_20/methanol 19:3:3) 7-O-[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]-5,6,7,4'-tetrahydroxyflavone **1** (4 mg, R_f 0.33).

GC/MS analysis of petroleum ether essential oil fraction (Ws1): A sample of petroleum ether essential oil extract (Ws1) was analyzed using GC/MS to give decane 2 (R_t 11.47 min, 5.69%), undecane 3 (R_t 15.33 min, 6.30%), dodecane 4 (R_t 18.96 min, 6.92%), undecane,2,6-dimethyl 5 (R_t 19.34 min, 1.63%), tridecane 6 (R_t 22.39 min, 4.24%), tetradecane 7 (R_t 25.66 min, 4.44%), pentadecane 8 (R_t 28.75 min, 5.55%), hexadecane 9 (R_t 31.71 min, 1.51%), tetradecane, 2,6,10-trimethyl 10 (R_t 34.65 min, 0.85%), nonadecane 11 (R_t 39.59 min, 3.53%), hexadecanoic acid methyl ester 12 (R_t 40.14 min, 1.73%), eicosane 13 (R_t 41.86 min, 3.34%), heneicosane 14 (R_t 44.10 min, 2.78%), phytol 15 (R_t 44.48 min, 3.04%), docosane 16 (R_t 46.22 min, 2.73%), tricosane 17 (R_t 48.25 min, 2.16%), tetracosane 18 (R_t 50.22 min, 1.58%), pentacosane 19 (R_t 52.12 min, 1.00%).

GC/MS analysis of methylene chloride essential oil fraction (Ws2): A sample of methylene chloride essential oil extract (Ws2) was analyzed using GC/MS analysis to give tetrahydrothiophene-1,1-dioxide 20 (R_t 19.58 min, 11.07%), heptadecane 21 (R_t 34.04 min, 2.79%), 2-methylheptadecane 22 (R_t 35.69 min, 0.28%), octadecane 23 (R_t 36.67 min, 3.50%), 2-methyloctadecane 24 (R_t 38.23 min, 0.53%), 9,12,15-octadecatrienoic acid methyl ester 25 (R_t 43.96 min, 0.53%), hexacosane 26 (R_t 53.94 min, 0.53%).

GC/MS analysis of hexane fraction (Ws3): A sample of hexane extract (Ws3) was analyzed using GC/MS analysis to give hexadecane 27 (R_t 31.15 min, 0.62%), octadecene 28 (R_t 36.51 min, 1.48%), 3,7,11-trimethyl-1-dodecanol 29 (R_t 39.72 min, 1.32%), 1-docosene 30 (R_t 41.41 min, 1.79%), octadecanoic acid 31 (R_t 41.54 min, 1.23%), 9,12-octadecadienoic acid (z,z)-methyl ester 32 (R_t 43.80 min, 21.80%), (z)-9-octadecenoic acid methyl ester 33 (R_t 43.95 min, 25.12%), (E)-9-octadecenoic acid methyl ester 34 (R_t 44.06 min, 0.27%), octadecanoic acid methyl ester 35 (R_t 44.52 min, 1.10%), 1-docosanol 36 (R_t 45.88 min, 1.05%), 1-eicosene 37 (R_t 45.99 min, 0.49%), heneicosene 38 (R_t 48.11 min, 0.46%), Cis-11-eicosenoic acid methyl ester 39 (R_t 48.23 min, 1.48%),

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eicosanoic acid methyl ester 40 (R_t 48.78 min, 0.41%), 13-docosenoic acid methyl ester 41 (R_t 52.23 min, 0.75%), docosanoic acid methyl ester 42 (R_t 52.72 min, 0.31%).

Table 2: MS data of compounds identified by GC/MS analyses (m/z [identity] (rel. abund. %)

Comp. #	Compound name	M.F	MS Data: m/z [identity] (rel. abund., %)
2	decane	$C_{10}H_{22}$	142 $[M^+]$ (8.66), 99 $[C_7H_{15}]^+$ (8.88), 85 $[C_6H_{13}]^+$ (28.88), 71 $[C_5H_{11}]^+$ (41.11), 57$[C_4H_9]^+$ (100).
3	undecane	$C_{11}H_{24}$	156 $[M^+]$ (6.66), 127 $[C_9H_{19}]^+$ (3.33), 113 $[C_8H_{17}]^+$ (5.55), 99 $[C_7H_{15}]^+$ (6.66), 85 $[C_6H_{13}]^+$ (31.11), 71 $[C_5H_{11}]^+$ (53.33), 57 $[C4H_9]^+$ (100).
4	dodecane	$C_{12}H_{26}$	170 $[M^+]$ (7.77), 141 $[C_{10}H_{21}]^+$ (2.22), 127 $[C_9H_{19}]^+$ (4.44), 99 $[C_7H_{15}]^+$ (7.77), 85 $[C_6H_{13}]^+$ (35.55), 71 $[C_5H_{11}]^+$ (58.88), 57 $[C_4H_9]^+$ (100).
5	2,6-dimethylundecane	$C_{13}H_{28}$	184 $[M^+]$ (1.11), 141 $[C_{10}H_{21}]^+$ (3.33), 113$[C_8H_{17}]^+$ (7.77), 99 $[C_7H_{15}]^+$ (7.77), 71 $[C_5H_{11}]^+$ (48.88), 57 $[C_4H_9]^+$ (100).
6	tridecane	$C_{13}H_{28}$	184 $[M^+]$ (6.66), 155 $[C_{11}H_{23}]^+$ (0.88), 127 $[C_9H_{19}]^+$ (4.44), 99 $[C_7H_{15}]^+$ (8.88), 85 $[C_6H_{13}]^+$ (37.77), 71 $[C_5H_{11}]^+$ (64.44), 57 $[C_4H_9]^+$ (100).
7	tetradecane	$C_{14}H_{30}$	198 $[M^+]$ (5.55), 169 $[C_{12}H_{25}]^+$ (0.88) , 141 $[C_{10}H_{21}]^+$ (13.33), 113 $[C_8H_{17}]^+$ (5.55), 99 $[C_7H_{15}]^+$ (11.11), 85 $[C_6H_{13}]^+$ (40), 71 $[C_5H_{11}]^+$ (65.55), 57 $[C_4H_9]^+$ (100).
8	Pentadecane	$C_{15}H_{32}$	212 $[M^+]$(4.44),155 $[C_{11}H_{23}]^+$ (10), 141 $[C_{10}H_{21}]^+$ (4.44), 113 $[C_8H_{17}]^+$ (6.88), 99 $[C_7H_{15}]^+$ (13.33), 85 $[C_6H_{13}]^+$ (43.33), 71 $[C_5H_{11}]^+$ (67.55), 57 $[C_4H_9]^+$ (100).
9	hexadecane	$C_{16}H_{34}$	226 $[M^+]$ (4.44, 183 $[C_{13}H_{27}]^+$ (1.11), 155 $[C_{11}H_{23}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (7.77), 99 $[C_7H_{15}]^+$ (15.55), 85 $[C_6H_{13}]^+$ (45.55), 71 $[C_5H_{11}]^+$ (67.11) , 57 $[C_4H_9]^+$ (100).
10	2,6,10-trimethyltetradecane	$C_{17}H_{36}$	240 $[M^+]$ (1.69), 183 $[C_{13}H_{27}]^+$ (5.97), 155 $[C_{11}H_{23}]^+$ (13.43), 127 $[C_9H_{19}]^+$ (4.47) , 85 $[C_6H_{13}]^+$ (50.74), 71 $[C_5H_{11}]^+$ (64.179), 57 $[C_4H_9]^+$ (100).
11	nonadecane	$C_{19}H_{40}$	268 $[M^+]$(3.33), 225 $[C_{16}H_{33}]^+$ (0.44), 211 $[C_{15}H_{31}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (2.66), 155 $[C_{11}H_{23}]^+$ (4.22), 141 $[C_{10}H_{21}]^+$ (6.66), 127 $[C_9H_{19}]^+$ (7.55), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (71.11), 57 $[C_4H_9]^+$ (100).
12	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270 $[M^+]$(6.66), 227 $[C_{14}H_{27}O_2]^+$ (5.55), 199 $[C_{12}H_{23}O_2]^+$ (3.55), 171 $[C_{10}H_{19}O_2]^+$ (4.22), 143 $[C_8H_{15}O_2]^+$ (14.44), 129 $[C_7H_{13}O_2]^+$ (6.88), 97 $[C_7H_{13}]^+$ (9.11), 87 $[C_4H_7O_2]^+$ (62.22), 73 $[C_3H_5O_2]^+$ (100).
13	eicosane	$C_{20}H_{42}$	282 $[M^+]$(3.11), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 169 $[C_{12}H_{25}]^+$ (2.88), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (0.88), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (15.77), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
14	heneicosane	$C_{21}H_{44}$	296 $[M^+]$ (2.22), 253 $[C_{18}H_{37}]^+$ (0.44), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (3.55), 144 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (7.77), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{13}]^+$ (50), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
15	phytol	$C_{20}H_{40}O$	278 $[C_{20}H_{38}]^+$ (0.44), 123 $[C_9H_{15}]^+$ (26.66), 95 $[C_7H_{11}]^+$ (17.77), 71 $[C_5H_{11}]^+$ (100).

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16	docosane	$C_{22}H_{46}$	310 [M+](1.33), 281 $[C_{20}H_{41}]^+$ (0.22), 253 $[C_{18}H_{37}]^+$ (0.66), 225 $[C_{16}H_{33}]^+$ (0.88), 197 $[C_{14}H_{29}]^+$ (1.11), 169 $[C_{12}H_{25}]^+$ (2.88), 141 $[C_{10}H_{21}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (10), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (71.11), 57 $[C_4H_9]^+$ (100).
17	tricosane	$C_{23}H_{48}$	324 $[M^+]$ (0.88), 281 $[C_{20}H_{41}]^+$ (0.22), 253 $[C_{18}H_{37}]^+$ (0.44), 225 $[C_{16}H_{33}]^+$ (0.66), 197 $[C_{14}H_{29}]^+$ (1.11), 169 $[C_{12}H_{25}]^+$ (2.44), 141 $[C_{10}H_{21}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{31}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
18	tetracosane	$C_{24}H_{50}$	338 $[M^+]$ (1.11), 281 $[C_{20}H_{41}]^+$ (0.44), 253 $[C_{18}H_{37}]^+$ (0.88), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.13), 169 $[C_{12}H_{25}]^+$ (3.11), 141 $[C_{10}H_{21}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{31}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
19	pentacosane	$C_{25}H_{52}$	352 $[M^+]$ (1.11), 309 $[C_{22}H_{45}]^+$ (0.22), 281 $[C_{20}H_{41}]^+$ (0.22), 253 $[C_{18}H_{37}]^+$ (0.66), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 169 $[C_{12}H_{25}]^+$ (2.66), 141 $[C_{10}H_{21}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (18.88), 85 $[C_6H_{31}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
20	tetrahydrothiophene - 1,1-dioxide	$C_4H_8O_2S$	120 $[M^+]$ (48.38), 93 $[C_2H_5O_2S]^+$ (1.61), 78 $[CH_3O_2S]^+$ (3.22), 55 $[C_4H_7]^+$ (100).
21	heptadecane	$C_{17}H_{36}$	240 $[M^+]$ (2.44), 197 $[C_{14}H_{29}]^+$ (0.55), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (2.22), 141 $[C_{10}H_{21}]^+$ (3.33), 127 $[C_9H_{19}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (8.88), 99 $[C_7H_{15}]^+$ (15.55), 85 $[C_6H_{13}]^+$ (46.66), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
22	2-methylheptadecane	$C_{18}H_{38}$	254 $[M^+]$(0.22), 211 $[C_{15}H_{31}]^+$ (6.66), 183 $[C_{13}H_{27}]^+$ (3.33), 155 $[C_{11}H23]^+$ (5.33), 141 $[C_{10}H_{21}]^+$ (8), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (46.66), 71 $[C_5H_{11}]^+$ (64.44), 57 $[C_4H_9]^+$ (100).
23	octadecane	$C_{18}H_{38}$	254 $[M^+]$ (2.22), 225 $[C_{16}H_{33}]^+$ (0.22), 197 $[C_{14}H_{29}]^+$ (1.11), 169 $[C_{12}H_{25}]^+$ (1.33), 155 $[C_{11}H_{23}]^+$ (3.11), 141 $[C_{10}H_{21}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (8.88), 99 $[C_7H_{15}]$+ (17.77), 85 $[C_6H_{13}]^+$ (45.33), 71 $[C_5H_{11}]^+$ (71.11), 57 $[C_4H_9]^+$ (100).
24	2-methyloctadecane	$C_{19}H_{40}$	268 $[M^+]$ (0.22), 253 $[C_{18}H_{37}]^+$ (0.88), 225 $[C_{16}H_{33}]^+$ (6.66), 197 $[C_{14}H_{29}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (4.44), 155 $[C_{11}H_{23}]^+$ (5.55), 141 $[C_{10}H_{21}]^+$ (7.77), 113 $[C_8H_{17}]^+$ (11.11) , 99 $[C_7H_{15}]^+$ (16.88), 85 $[C_6H_{13}]^+$ (43.33), 71 $[C_5H_{11}]^+$ (62.22), 57 $[C_4H_9]^+$ (100).
25	9,12,15-octadecatrienoic acid methyl ester	$C_{19}H_{32}O_2$	292 $[M^+]$ (2.22), 264 $[C_{17}H_{28}O_2]^+$ (1.11), 237 $[C_{15}H_{25}O_2]^+$ (1.11), 191 $[C_{14}H_{23}]^+$ (3.33), 165 $[C_{12}H_{21}]^+$ (4.44), 149 $[C_{11}H_{17}]^+$ (10), 121 $[C_9H_{13}]^+$ (17.77), 108 $[C_8H_{12}]^+$ (35.55), 95 $[C_7H_{11}]^+$ (62.22), 79 $[C_6H_7]^+$ (100), 67 $[C_5H_7]^+$ (73.33), 55 $[C_4H_7]^+$ (83.33).
26	hexacosane	$C_{26}H_{54}$	366 $[M^+]$ (0.88), 309 $[C_{22}H_{45}]^+$ (0.22), 281 $[C_{20}H_{24}]^+$ (1.11), 253 $[C_{18}H_{37}]^+$ (1.11), 225 $[C_{16}H_{33}]^+$ (0.88), 197 $[C_{14}H_{29}]^+$ (1.33), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (3.55), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (7.77), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]$+ (17.77), 85 $[C_6H_{13}]^+$ (46.66), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
27	hexadecane	$C_{16}H_{32}$	224 $[M^+]$ (1.11), 167 $[C_{12}H_{23}]^+$ (2.22), 139 $[C_{10}H_{19}]^+$, 125 $[C_9H_{17}]^+$ (13.3), 111 $[C_8H_{15}]^+$ (32.22), 97 (4.44) $[C_7H_{13}]^+$ (65.55), 83 $[C_6H_{11}]^+$ (82.22), 55 $[C_4H_7]^+$ (100).
28	octadecene	$C_{18}H_{36}$	252 $[M^+]$ (1.11), 223 $[C_{16}H_{31}]^+$ (1.11), 195 $[C_{14}H_{27}]^+$ (1.11), 167 $[C_{12}H_{23}]^+$ (2.22), 153 $[C_{11}H_{21}]^+$ (3.33), 139 $[C_{10}H_{19}]^+$ (5.55), 125 $[C_9H_{17}]^+$ (16.66), 111 $[C_8H_{15}]^+$ (37.77), 97 $[C_7H_{13}]^+$ (81.11), 83 $[C_6H_{11}]^+$ (87.77), 69 $[C_5H_9]^+$ (75.55), 55 $[C_4H_7]^+$ (100).
29	1-dodecanol, 3,7,11-trimethyl	$C_{15}H_{32}O$	228 $[M^+]$ (1.17), 157 $[C_{10}H_{21}O]^+$ (1.17), 149 (7.05), 125 $[C_9H_{17}]^+$ (29.41), 111 $[C_8H_{15}]^+$ (22.35), 97 $[C_7H_{13}]^+$ (57.64), 84 $[C_6H_{12}]^+$ (98.82), 55 $[C_4H_7]^+$ (100).
30	1-docosene	$C_{22}H_{44}$	308 $[M^+]$ (1.14), 223 $[C_{16}H_{31}]^+$ (1.14), 195 $[C_{14}H_{27}]^+$ (2.29), 167 $[C_{12}H_{23}]^+$ (2.22), 153 $[C_{11}H_{21}]^+$ (3.44), 139 $[C_{10}H_{19}]^+$ (6.89), 125 $[C_9H_{17}]^+$ (20.68), 111 $[C_8H_{15}]^+$ (45.97), 97 $[C_7H_{13}]^+$ (89.65), 83

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			$[C_6H_{11}]^+$ (95.40), 69 $[C_5H_9]^+$ (83.90), 55 $[C_4H_7]^+$ (100).
31	octadecanoic acid	$C_{18}H_{36}O_2$	284 $[M^+]$ (1.11), 239 $[C_{17}H_{35}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.11), 169 $[C_{12}H_{25}]^+$ (2.22), 141 $[C_{10}H_{21}]^+$ (3.33), 113 $[C_8H_{17}]^+$ (8.88), 99 $[C_7H_{15}]$+ (13.33), 85 $[C_6H_{13}]^+$ (44.44), 71 $[C_5H_{11}]^+$ (66.66), 57 $[C_4H_9]^+$ (100).
32	(z,z) 9,12-octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294 $[M^+]$ (4.49), 263 $[C_{18}H_{31}O]^+$ (2.24), 149 $[C_{11}H_{17}]^+$ (6.74), 123 $[C_9H_{15}]^+$ (11.23), 109 $[C_8H_{13}]^+$ (24.71), 95 $[C_7H_{11}]^+$ (53.93), 81 $[C_6H_9]^+$ (79.77), 67 $[C_5H_7]^+$ (100), 55 $[C_4H_7]^+$ (64.04).
33	(z)9-octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296 $[M^+]$(2.22), 265 $[C_{18}H_{33}O]^+$ (5.55), 222 $[C_{16}H_{30}]^+$ (6.66), 180 $[C_{13}H_{24}]^+$ (7.77), 137 $[C_{10}H_{17}]^+$ (7.77), 111 $[C_8H_{15}]^+$ (20), 97 $[C_7H_{13}]^+$ (41.11), 83 $[C_6H_{11}]^+$ (48.88), 69 $[C_5H_9]^+$ (62.22), 55 $[C_4H_7]^+$ (100).
34	(E)9-octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296 $[M^+]$ (1.11), 265 $[C_{18}H_{33}O]^+$ (3.33), 222 $[C_{16}H_{30}]^+$ (4.44), 180 $[C_{13}H_{24}]^+$ (5.55), 137 $[C_{10}H_{17}]^+$ (6.66), 111 $[C_8H_{15}]^+$ (16.66), 97 $[C_7H_{13}]^+$ (37.77), 83 $[C_6H_{11}]^+$ (42.22), 69 $[C_5H_9]^+$ (63.33), 55 $[C_4H_7]^+$ (100).
35	octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298 $[M^+]$ (5.61), 255 $[C_{16}H_{31}O_2]^+$ (3.37), 227 $[C_{14}H_{27}O_2]^+$ (1.12), 199 $[C_{12}H_{23}O_2]^+$ (4.49), 157 $[C_9H_{17}O_2]^+$ (2.24), 143 $[C_8H_{15}O_2]^+$ (14.60), 129 $[C_7H_{13}O_2]^+$ (5.61), 97 $[C_7H_{13}]^+$ (11.23), 87 $[C_4H_7O_2]^+$ (61.79), 74 $[C_3H_6O_2]^+$ (100), 55 $[C_4H_7]^+$ (35.95).
36	1-docosanol	$C_{22}H_{46}O$	268 $[M^+]$ (1.12),281 $[C_{20}H_{41}]^+$ (1.12), 167 $[C_{12}H_{23}]^+$ (3.37), 153 $[C_{11}H_{21}]^+$ (4.49), 139 $[C_{10}H_{19}]^+$ (8.98), 125 $[C_9H_{17}]^+$ (20.22), 111 $[C_8H_{15}]^+$ (40.44),98 $[C_7H_{14}]^+$ (19.10), 83 $[C_6H_{11}]^+$ (89.88), 69 $[C_5H_9]^+$ (75.28), 57 $[C_4H_9]^+$ (100).
37	1-eicosene	$C_{20}H_{40}$	280 $[M^+]$ (1.11), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (4.44), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]$+ (16.66), 85 $[C_6H_{13}]^+$ (51.11), 71 $[C_5H_{11}]^+$ (72.22), 57 $[C_4H_9]^+$ (100).
38	heneicosene	$C_{21}H_{42}$	294 $[M^+]$ (1.11), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (10), 141 $[C_{10}H_{21}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (14.44), 99 $[C_7H_{15}]$+ (16.66), 85 $[C_6H_{13}]^+$ (41.11), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
39	cis-11-eicosenoic acid methyl ester	$C_{21}H_{40}O_2$	324 $[M^+]$ (1.12),292 $[C_{20}H_{36}O]^+$ (7.86), 250 $[C_{18}H_{34}]^+$ (4.49), 167 $[C_{12}H_{23}]^+$ (3.37), 125 $[C_9H_{17}]^+$ (10.11), 111 $[C_8H_{15}]^+$ (20.22),97 $[C_7H_{13}]^+$ (46.06), 83 $[C_6H_{11}]^+$ (52.80), 69 $[C_5H_9]^+$ (69.66), 55 $[C_4H_7]^+$ (100).
40	eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	326 $[M^+]$ (6.74), 283 $[C_{18}H_{35}O_2]^+$ (3.37), 255 $[C_{16}H_{31}O_2]^+$ (1.12), 227 $[C_{14}H_{27}O_2]^+$ (2.24), 199 $[C_{12}H_{23}O_2]^+$ (4.49), 157 $[C_9H_{17}O_2]^+$ (3.37), 143 $[C_8H_{15}O_2]^+$ (15.73), 87 $[C_4H_7O_2]^+$ (67.41), 74 $[C_3H_6O_2]^+$ (100), 55 $[C_4H_7]^+$ (52.80).
41	13-docosenoic acid methyl ester	$C_{23}H_{44}O_2$	352 $[M^+]$ (1.12), 320 $[C_{22}H_{40}O]^+$ (7.86), 278 $[C_{20}H_{38}]^+$ (2.24), 236 $[C_{17}H_{32}]^+$ (2.24), 207 $[C_{15}H_{27}]^+$ (4.49), 166 $[C_{12}H_{22}]^+$ (3.37), 111 $[C_8H_{15}]^+$ (20.22), 97 $[C_7H_{13}]^+$ (42.69), 83 $[C_6H_{11}]^+$ (51.68), 69 $[C_5H_9]^+$ (75.28), 55 $[C_4H_7]^+$ (100).
42	docosanoic acid methyl ester	$C_{23}H_{46}O_2$	354 $[M^+]$ (8.98), 311 $[C_{20}H_{29}O_2]^+$ (3.37), 283 $[C_{18}H_{35}O_2]^+$ (1.12), 255 $[C_{16}H_{31}O_2]^+$ (3.37), 199 $[C_{12}H_{23}O_2]^+$ (4.49), 157 $[C_9H_{17}O_2]^+$ (5.61), 143 $[C_8H_{15}O_2]^+$ (21.34), 129 $[C_7H_{13}O_2]^+$ (14.60), 115 $[C_6H_{11}O_2]^+$ (4.49), 87 $[C_4H_7O_2]^+$ (77.52), 74 $[C_3H_6O_2]^+$ (100), 55 $[C_4H_7]^+$ (75.28).

Antimicrobial activity assessment:

Extracts were individually tested against Gram positive *Staphylococcus aureus*, Gram negative *Escherichia coli* bacterial and fungi *Candida albicans*. Each of the compounds was dissolved in DMSO and a solution of concentration 1 mg /ml were applied separately to paper discs of Whatman filter with standard size (5mm), cut and sterilized in an autoclave. The paper discs were soaked in the desired concentration of the extract solution and placed aseptically in the Petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with *Staphylococcus aureus*, *E. coli* and *Candida albicans*. The Petri dishes

were incubated at 36^{0}C and the inhibition zones were recorded after 24h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic Ampicillin, *Gentamicin* and Antifungal *Amphotericin* B was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the extract was calculated by the formula as under:

$$\%\ Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ extract\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre) x\ 100}$$

Free radical scavenging antioxidant activity (DPPH):

The antioxidant activity of extract was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University by the DPPH free radical scavenging assay in triplicate and average values were considered.

Freshly prepared (0.004% w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10^{0}C in the dark. A methanol solution of the test compound was prepared. A 40 uL aliquot of the methanol solution was added to 3ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = [\{(AC - AT)/ AC\} \times 100]$$

Where *A*C = Absorbance of the control at t = 0 min and *A*T = absorbance of the sample+DPPH at t = 16 min [22-24].

Cytotoxicity Assay

The cell lines HePG2, MCF-7and HCT-116 were used to determine the inhibitory effects of extracts on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. HepG2 was cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37^{0}C in a 5% CO_2 incubator. The cell line was seedes in a 96-well plate at a density of 1.0x104 cells/well. [25] at 37^{0}C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of extract treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

CONCLUSION

Wisteria sinensis exhibited various anticancer, antioxidants, antimicrobial activities. The chromatographic separation of extracts led to a new flavone glycoside, 7-O-[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]-5,6,7,4'-tetrahydroxy flavone, in addition to many known compounds from the volatile fractions by GC/MS analysis.

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