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Phytochemical and biological study of *Chorisia speciosa* A. St. Hil. Cultivated in Egypt

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Abstract
Chromatographic fractionation of the alcoholic extract of leaves of *Chorisia speciosa* A. St. Hil. (Bombacaceae) resulted in the isolation and identification of ten compounds, two of them are firstly reported in the family: β-amyrin (1), and Verbascoside (7), in addition to five compounds firstly isolated from the plant p-hydroxy benzoic acid (3), β-sitosterol-3-O-β-d-glucopyranoside (4), succinic acid (5), astragalin (8) and cinaroside (9), in addition to β-sitosterol (2), tiliroside (6) and rhiofolin (10).
Their structures were established by various spectroscopic methods (^{1}H NMR, 13C NMR, HSQC, HMBC and UV spectroscopy) as well as comparison with authentic samples.
Biological studies of different extracts of *Chorisia speciosa* A. St. Hil. showed that the extracts have slightly higher antioxidant activity.

Keywords: *Chorisia speciosa* A. St. Hil, anti-inflammatory, antioxidant

Introduction

Chorisia speciosa A. St. Hil is a member of the family Bombacaceae (Bombax, Baobab or Kapok family), which is a plant family of flowering plants, contains about 28 genera and about 200 species [1].

Plants of this family are perennial, deciduous and woody trees. They occur throughout the tropical and subtropical regions of the world especially in tropical America [2].

In Egypt, Bombacaeae is represented by two genera, *Bombax* and *Chorisia* which are cultivated mostly for ornamental and shade purposes due to their large branches and brightly colored flowers [3].

Chorisia is the name of a genus of about 20 species of large trees found in tropical and subtropical areas, including Mexico, Central America, South America, the Bahamas, the Caribbean, West Africa, and Southeast Asia. It was named in honor of the botanical artist and traveler Ludwig L. Choris (1795 - 1828, 19th century) [4].

Chorisia is mainly cultivated for its ornamental brilliant flowers since it blooms during autumn, adding a touch of color at the time when most blooms are fading. It is also cultivated for the silky fiber (or floss) that is obtained from the ripened seeds, so named as "silk floss tree". Additionally, because of its twisted shape, it is sometimes nicknamed as "the drunken tree" [4-5].

These plants are traditionally used for many health disorders, e.g., headache, fever, diabetes, diarrhea, parasitic infections, peptic ulcer and rheumatism [6].

Biologically, it was reported that some *Chorisia* species possess wide range of useful anti-inflammatory, hepatoprotective, cytotoxic, antioxidant and hypoglycemic with high safety margins [7-10].

Chorisia is characterized by a bottle-shaped trunk generally bulging in its lower third, measuring up to two meters in girth with thick conical sharp spines as an adaptation for dry times to store water. In young trees, the trunk is green due to its high chlorophyll content which makes it capable of performing photosynthesis when leaves are absent and with age, it turns to grey. Flowers usually appear when the tree is leafless [11].

The silk floss tree (*Ceiba speciosa* A. St.-Hil., formerly *Chorisia speciosa* A. St. Hil.), is a species of deciduous tree native to southern Brazil, Argentina, Bolivia, Paraguay, Peru and Ecuador, but it is also cultivated in many tropical areas and grows well in parts of southern California [4-5].

Materials and Methods Equipment

Melting points are carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). UV spectra were recorded in MeOH on Ultrospec 1000, UV-VIS spectrometer, Pharmacia Biotech, Cambridge, England. NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on JEOL ALPHA-500 spectrometer, Bruker Advanc III 400 MHz spectrophotometer (Bruker BioSpin AG, Fällanden, Switzerland), and Agilent INOVA 600AS instrument (600, Japan) using DMSO-d6, $CDCl_3$, and C_5D_5N as solvents. Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm, Merck), RP-18 (0.04–0.063 mm, Merck).

TLC was performed on precoated TLC plates silica gel 60 F254 (0.2 mm, Merck). The solvent systems used for TLC analysis include n-hexane: acetone (80:20, S1), CH_2Cl_2: MeOH (90:10, S2), CH_2Cl_2: MeOH (80:20, S3).

Plant Materials

The plant materials used in this study are the leaves, flowers, fruits and stem of *Chorisia speciosa*. A. St. Hil. They were collected during the flowering stage in the period of February to November 2012 from the fields of Faculty of Pharmacy at Assiut University. The plant was kindly identified and authenticated by late Prof. Dr. Naeem E. Keltawy, Professor of Ornamental Horticulture and Floriculture, Faculty of Agriculture, Assiut University.

Chemicals

DPPH (2,2-Diphenyl-1-picryl-hydrazine) and carrageenan were purchased from Aldrich Co., USA, tween 80% (Sigma Chemical Co., St. Louis, USA), Normal saline 0.9% (El-Nasr pharmaceutical and chemical Co., Egypt), Indomethacin (El-Nile Co., Egypt).

Extraction and isolation

The air-dried powdered leaves of C. Speciosa A, St. Hil (1 kg) were exhaustively extracted with 70% ethanol by maceration at room temperature.

The ethanolic extract was concentrated under reduced pressure to obtain a viscous residue (500 g). The concentrated ethanolic extract (500 g) was digested in a least amount of distilled water, transferred to a separating funnel and extracted with successive portions of n-hexane, chloroform, ethyl acetate and n-butanol. The n-hexane, chloroform, ethyl acetate and n-butanol washings were concentrated separately under reduced pressure to give 400, 7, 6 and 35 g respectively.

The n hexane fraction (400 g) was chromatographed on silica gel column using n-hexane: ethyl acetate in a gradient elution manner, where seven subfractions (HL-I-:HL-VII) were obtained. Subfraction HL-III (50 g) eluted with n-hexane-ethyl acetate (95:5) was chromatographed on silica gel column using n-hexane-acetone in a gradient elution manner to give compound 1.

Subfraction HL-IV (26 g) was subjected to silica gel CC using n- hexane-acetone in a gradient elution manner to give compound 2.

The chloroform fraction (7 g) was chromatographed on silica gel column using CH_2Cl_2-MeOH in a gradient elution manner, where six subfractions (CH-I: CHVI) were obtained. Subfraction CH-II (1.2 g, eluted with CH_2Cl_2-MeOH 98:2) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner to give compounds 3 and 4. Subfraction CH-III (1.3 g, eluted with CH_2Cl_2-MeOH 95:5) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner to give compound 5.

The EtOAC fraction (6 g) was subjected to silica gel CC using gradient system of CH_2Cl_2-MeOH, where seven subfractions (E-I: E-VII) were obtained. Subfraction E-III (1.2 g) eluted with CH_2Cl_2- MeOH (95:5) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner to give compound 6. Subfraction E-IV (1.5 g) eluted with CH_2Cl_2- MeOH (90:10) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner to give compounds 7 and 8. The subfraction E-V (0.9 g) eluted with (CH_2Cl_2- MeOH 85:15), was subjected to sephadex CC using MeOH in a gradient elution manner to give compound 9. The subfraction E-VI (1 g) eluted with (CH_2Cl_2- MeOH 80:20), was subjected to sephadex CC using MeOH in a gradient elution manner to give compound 10.

The n- butanol fraction (35 g) was subjected to silica gel CC using gradient system of CH_2Cl_2-MeOH, where six subfractions (Bu-I: Bu-VI) were obtained. Subfraction Bu-IV (10 g) eluted with CH_2Cl_2- MeOH (80:20) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner to give compound 10.

Acid hydrolysis

Few milligrams of compounds 6, 8 and 9 were separately dissolved in 5 ml MeOH and an equal volume of 10% H_2SO_4 v/v was added. The mixture was refluxed for 3 hrs, then cooled. The hydrolyzate was shaken with ethyl acetate 3 times, distilled off and the aglycone was subjected to TLC using $CHCl_3$: MeOH (85:15) as solvent system. The acidic solution was then neutralized with barium carbonate, concentrated and spotted alongside authentic sugars on Whatman No. 1 sheets using n- butanol-acetic acid-water (4:1:2, v/v/v) as a solvent
system.

Antioxidant activity

Antioxidant activity was determined by the DPPH method [12]. 0.2 ml of methanolic solutions of the total ethanolic leaf, stem bark and fruit extracts of *C. speciosa* A. St. Hil., also the leaf and stem bark fractions; including (n-hexane, chloroform, ethyl acetate and n-butanol) and also rhiofolin, a flavonoid isolated from n- butanol fraction (0.0625, 0.125, 0.25, 0.5, 1 mg/ml). were mixed with 2ml of methanolic solution of DPPH· (100 µM). Similarly 0.2 ml of methanolic solutions of quercetin in a concentration range of (1000-5 µg/ml) is mixed with 2 ml DPPH· and used as a positive control.

A mixture of 0.2 ml of methanol and 2 ml of methanolic solution of DPPH· (100 µM) served as blank. After mixing, all the solutions were incubated in dark for 30 minutes and absorbance was measured at 517 nm. The experiment was performed in triplicates and percent of antioxidant activity was calculated as follows:

$$\text{Antioxidant activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Pharmacological study Animals

Adult male albino rats (each 100-120 g) were used. All animal procedures were conducted in accordance with the Good Laboratory Practice (GLP) according to internationally valid guidelines and regulations of the World Health Organization (WHO) [13, 14].

Preparation of extracts for administration
Weighed amount (1 g) of each extract was separately taken and solubilized in 3% (v/v) tween 80 in normal saline and the volume was completed to 10 ml by normal saline. A control solution was prepared using the same amount of tween 80 in normal saline (negative control).

Acute toxicity
The acute toxicity tests (LD_{50}) of the total ethanolic extracts of leaves, stem bark and fruits were determined according to the procedure described by [15]. The animals were divided into 3 groups (3 rats/group) for each given dose. Doses of 1, 2 and 3 g/kg were administered intraperitoneally (i.p.).

Anti-inflammatory activity
Carrageenan-induced rat hind paw edema model described by[16] was performed, where ten groups (6 rats/ group) were used. The first group was kept as a negative control, injected intraperitoneally by 3% tween 80 in normal saline, while the second group injected by indomethacin (8mg/Kg) as a positive control. The other groups were separately intraperitioneally injected with different extracts at a dose of 400 mg/kg of the body weight. After 30 minutes from administration, the inflammation was induced by injection of the carrageenan suspension in the right paw while the left one was injected by an equal volume of saline solution. The percentage of inhibition was calculated.

Antipyretic activity (17, 18)
Fourteen groups of (6 rats/group) were used and the rectal temperature was recorded with a thermometer. Hyperthermia was induced at first by subcutaneous injection 20% (w/v) aqueous suspension of yeast in a volume of
10 ml/kg. The first group was kept as a negative control injected intraperitoneally by 2% tween 80 in 0.9 normal saline. While the second group injected by indomethacine (8 mg/kg) as a positive control. The other groups were separately injected intraperitoneally with the different fractions of leaves, stem and fruits and also with a pure isolated flavonoid, rhiofolin at doses of 400 mg/kg body weight. Rectal temperatures were taken after 1, 2, 3, 4 and 5 hrs from administration of tested fractions.

Statistical analysis
Data were analyzed using student's T-test and the values were expressed as mean ± S.E. (n = 6 animals).

Results
Ten compounds were isolated from the ethanolic leaves extract of *C. speciosa* A. St. Hil.

Compound 1
White fine needles (40 mg, acetone), m.p. 198- 200 °C; R_f 0.81 (S1). Mixed melting point as well as cochromatography with authentic sample, compound 1 was identified as *β*-amyrin.

Compound 2
White needles (170 mg, acetone), m.p. 168-170 °C; R_f 0.61 (S1). Mixed melting point as well as cochromatography with authentic sample, compound 2 was identified as stigmasterol.

Compound 3
Faint yellow crystal (25 mg, MeOH)), m.p. 213-217 °C; Rf 0.51 (S2); 1HNMR ($CDCl_3$, 400 MHz): δH 8.14 (2H, d, J = 8.0 Hz, H-2,6), 6.91 (2H, d, J = 8.0 Hz, H-3,5), ^{13}C-NMR ($CDCl_3$, 100 MHz): δC 129.29 (C-1), 126.69 (C-2,6), 116.14 (C-3,5), 162.52 (C-4), 170.00 (C-7), By comparison with the reported data [19,20], compound 3 was identified as *p*-hydroxy benzoic acid.

Compound 4
White powder (100 mg, MeOH)), m.p. 290-292 °C; R_f 0.46 (S2); Mixed melting point as well as cochromatography with authentic sample, By comparison with the reported data[3,21], compound 4 was identified as *β*-sitosterol-3-*O*-*β*-D-glucoside.

Compound 5
White needles (32 mg, MeOH)), m.p. 180-182 °C; R_f 0.61 (S2); 1HNMR (CD_3OD, 400 MHz): δH 2.59 (4H, s, H-2, 3), ^{13}C-NMR (CD_3OD, 100 MHz): δC 28.75 (C-2, 3), 175.08 (C-1,4), By comparison with the reported data [10], compound 5 was identified as succinic acid.

Compound 6
Yellow brown powder (60 mg, MeOH), m.p. 257- 260ºC; R_f 0.60 (S3); UV (MeOH): λmax 269, 330; +NaOMe: 275, 369; +AlCl3: 276, 394; +$AlCl_3$/HCl: 275, 394; +NaOAc: 275, 330; +NaOAc/H_3BO_3: 269, 330 nm; ^{1}HNMR (CD_3OD, 600 MHz): δH 3.14-3.60 (5H, m, sugar protons), 4.3 (1H, d, J=7.8 Hz, H-1''), 6.13 (1H, br.s, H-6), 6.31 (1H, br.s, H-8), 7.32 (2H, d, J=7.8 Hz, H-2',6'), 6.68 (2H, d, J=7.8 Hz, H-3',5'), 7.99 (2H, d, J=8.4 Hz, H-2''',6'''), 6.82 (2H, d, J=8.4 Hz, H-3''',5'''), 7.68 (1H, d, J= 15.6 Hz, H-7'''), 6.37 (1H, d, J= 15.6 Hz, 8'''); 13C-NMR (CD_3OD, 150 MHz): δC 154.17 (C-2), 132.61 (C-3), 167.87 (C-4), 161.40 (C-5), 102.54 (C-6), 164.68 (C-7), 100.75 (C-8), 158.05 (C-9), 111.35 (C-10), 123.92 (C-1'), 130.70 (C-2',6'), 115.89 (C-3',5'), 160.78 (C-4'), 127.19 (C-1'''), 131.74 (C-2''',6'''), 116.69 (C-3''',5'''), 160.62 (C-4'''), 147.50 (C-7'''), 114.63 (C-8'''), 167.63 (C-9'''), 103.76 (C-1''), 73.33 (C-2''), 75.57 (C-3''), 71.84 (C-4''), 75.77 (C-5''), 61.89 (C-6''). By comparison with the reported data[8,10,22,23], compound 6 was identified as Kaempferol 3-*O*-*β*-D-(6''-*E*-*p*-coumaroyl)- glucopyranoside.

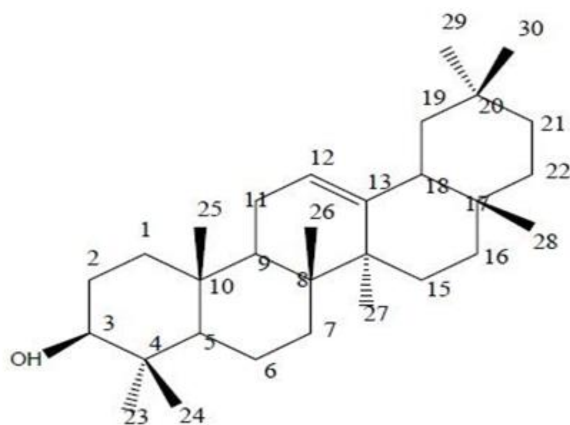
Compound 7
Yellowish brown amorphous powder (40 mg, MeOH), R_f 0.56 (S3); ^{1}HNMR (CD_3OD, 400 MHz): δH 3.3-3.9 (9H, m, sugar protons), 4.33 (1H, d, J= 8.0, H-1'), 5.14 (1H, br.s, H-1''), 1.04 (3H, d, J= 6.4, H-6''), 6.65(1H, d, J= 2.0 Hz, H-2), 6.64 (1H, d, J= 8.0 Hz, H-5), 6.52 (1H, dd, J= 8.0, 2.0 Hz, H-6), 2.76
(2H, t, J= 8.4 Hz, H-7), (4.01 (1H, dd, J=8.0, 16.4 Hz), 3.66 (1H, dd, J=8.0, 16.4 Hz) H-8), 7.01 (1H, d, J= 2.0 Hz, H-2'''), 6.73 (1H, d, J= 8.0 Hz, H-5'''), 6.91 (1H, dd, J= 8.0, 2.0 Hz, H-6'''), 7.55 (1H, d, J= 16.0 Hz, H-7'''), 6.23 (1H, d, J=16.0 Hz, H-8'''); ^{13}C-NMR (CD_3OD, 150 MHz): δC 131.29 (C-1), 116.31 (C-2), 144.42 (C-3), 145.88 (C-4), 116.90 (C-5), 121.04 (C-6), 36.34 (C-7), 72.01 (C-8), 103.97 (C-1'), 75.98 (C-2'), 81.42 (C-3'), 70.40 (C-4'), 75.81 (C-5'), 62.16 (C-6'), 102.77 (C-1''), 72.13 (C-2''), 71.86 (C-3''), 73.59 (C-4''), 70.19 (C-5''), 18.23 (C-6''), 127.45 (C-1'''), 116.1 (C-2'''), 146.57 (C-3'''), 149.52 (C-4'''), 114.5 (C-5'''), 122.96 (C-6'''), 147.76 (C-7'''), 115.05 (C-8'''), 168.07 (C-9'''). By comparison with the reported data [24, 25], compound 7 was identified as 3.4-dihydroxyphenethyl alcohol-8-*O*-[(4'-*O*-caffeoyl)-*α*-L-rhamnopyranosyl - (1→3)]-*β*-D-glucopyranoside.

Compound 8
Yellow powder (75 mg, MeOH), m.p. 198-200ºC; R_f 0.54 (S3); UV (MeOH): λmax 267, 335; +NaOMe: 271, 389; +$AlCl_3$: 275, 383; +$AlCl_3$/HCl: 276, 380; +NaOAc: 274, 338;

+NaOAc/H_3BO_3: 268, 338 nm; ^{1}HNMR (DMSO, 400 MHz): δH 3.10-3.72 (5H, m, sugar protons), 5.08 (1H, d, J=7.2 Hz, H-1''), 6.23 (1H, br.s, H-6), 6.45 (1H, br.s, H-8), 7.96 (2H, d, J=8.4 Hz, H-2',6'), 6.96 (2H, d, J=8.4 Hz, H-3',5'); ^{13}C-NMR (DMSO, 100 MHz): δC 156.88 (C-2), 131.44 (C-3), 182.45 (C-4), 163.38 (C-5), 100.00 (C-6), 164.78 (C-7), 95.32 (C-8), 157.40 (C-9), 105.78 (C-10), 121.42 (C-1'), 129.09 (C-2',6'), 116.51 (C-3',5'), 161.61 (C-4'), 103.50 (C-1''), 73.53 (C-2''), 77.55 (C-3''), 70.01 (C-4''), 76.81 (C-5''), 61.05 (C-6''). By comparison with the reported data [10], compound 8 was identified as Kaempferol 3-*O*-*β*-D-glucopyranoside.

Compound 9
Yellow powder (20 mg, MeOH), m.p. 262-264ºC; R_f 0.52 (S3); UV (MeOH): λmax 267, 332; +NaOMe: 271, 371; +AlCl), 5.1 (1H, d, J=6.8 Hz, H-1''), 6.63 (1H, S, H-3), 6.52 (1H, d, J=2, H-6), 6.82 (1H, d, J=2, H-8), 7.43 (1H, d, J=2.0 Hz, H-2''), 6.94 (1H, d, J=8.0 Hz, H-5'), $_3$: 271, 385; +$AlCl_3$/HCl: 278, 350; +NaOAc: 268, 332; +NaOAc/H_3BO_3: 270, 356 nm; ^{1}HNMR (CD_3OD, 400 MHz): δH 3.19-3.71 (5H, m, sugar protons7.44 (1H, dd, J=8.0, 2.0, H-6'). By comparison with the reported data [26], compound 9 was identified as luteolin-7-
O-*β*-D-glucopyranoside.

Compound 10
Yellow powder (4 g, MeOH), m.p. 243-245ºC; Rf 0.36 (S3); UV (MeOH): λmax 269, 334; +NaOMe: 269, 388; +$AlCl_3$: 275, 382; +$AlCl_3$/HCl: 277, 380; +NaOAc: 269, 342; +NaOAc/H_3BO_3: 269, 336 nm; ^{1}HNMR (DMSO, 400 MHz): δH 3.19-3.60 (5H, m, sugar protons), 5.23 (1H, d, J=8.0 Hz, H-1''), 3.60-3.85 (5H, m, sugar protons), 5.13 (1H, s, H-1'''), 1.19 (3H, d, J=7.6, H-CH_3-6'''), 6.77 (1H, s, H-3), 6.34 (1H, d. J=1.6, H-6), 6.79 (1H, d, J=1.6, H-8), 7.87 (2H, d, J=8.0 Hz, H-2', 6'), 6.84 (2H, d, J=8.0 Hz, H-3', 5'); ^{13}C-NMR (DMSO, 100 MHz): δC 154.80 (C-2), 102.00 (C-3), 181.81 (C-4), 162.44 (C-5), 99.32 (C-6), 162.44 (C-7), 94.36 (C-8), 157.02 (C-9), 105.44 (C-10), 118.00 (C-1'), 128.69 (C-2',6'), 116.86 (C-3',5'), 161.30 (C-4'), 97.82 (C-1''), 77.08 (C-2''), 77.29 (C-3''), 70.46 (C-4''), 76.26 (C-5''), 60.50 (C-6''), 100.53 (C-1'''), 71.91 (C-2'''), 69.70 (C-3'''), 74.73 (C-4'''), 68.41 (C-5'''), 18.17 (C-6''' of CH_3). By comparison with the reported data [27], compound 10 was identified as Apigenin 7-*O*-neohesperidoside.



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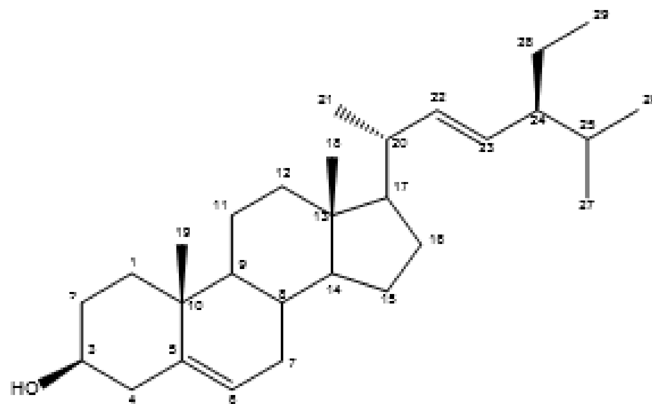

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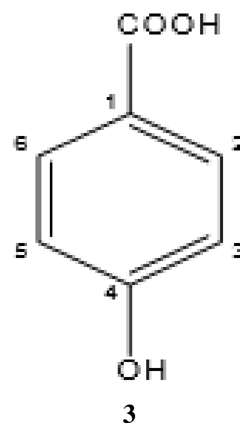

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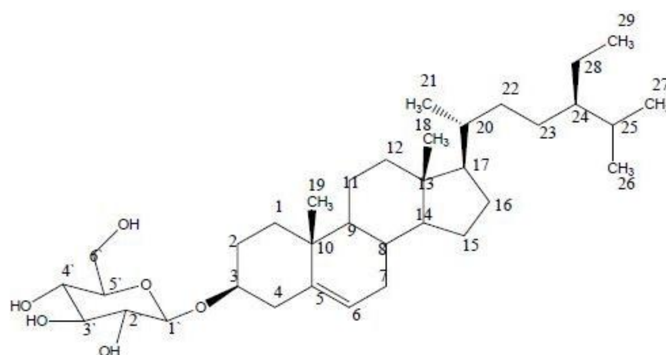

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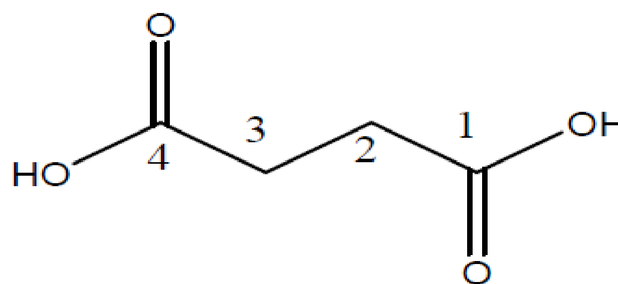

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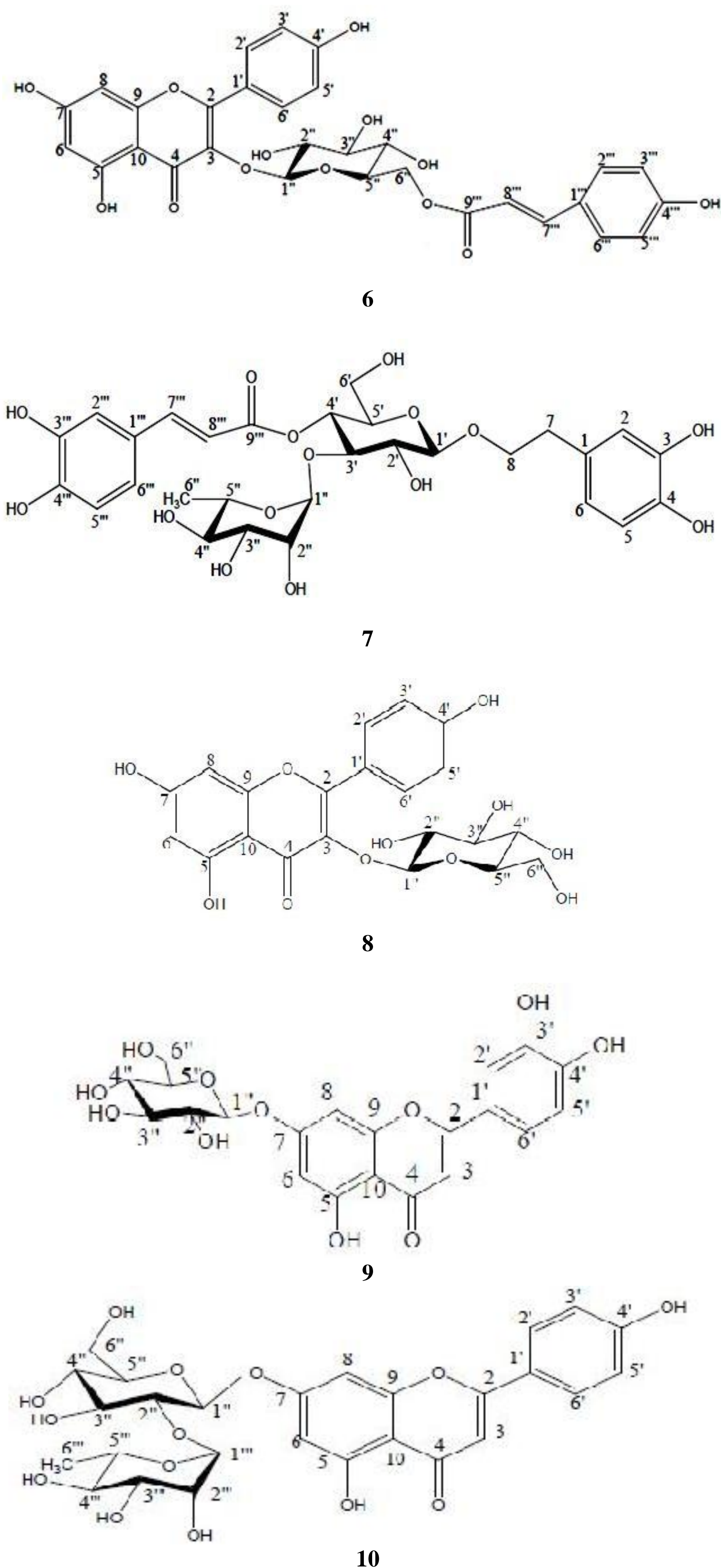

Fig 1: Structures of the isolated compounds (1-10) from *C. speciosa* A. St. Hil.

Biological Activity Antioxidant activity

All the tested extracts exhibited strong antioxidant activity especially in ethyl acetate and n-butanol extracts of leaf, ethyl acetate extract of stem and total ethanolic extract of fruits, The results are listed in Table 1.

Pharmacological study Acute toxicity

No signs of toxicity were observed in rats given the total ethanolic extracts of leaves, stem bark and fruits at the chosen doses level (3 g/kg).

Anti-inflammatory activity

All the tested extracts possess anti-inflammatory activity in carrageenin induced rat hind paw oedema model when the animals were pretreated with the different extracts 30 min before injecting the phlogistic agent. The effects begin within the first hour becoming highly significant in the 2nd, 3rd and 4th hours and continue till the 5th hour in the total ethanolic extract of stem and the chloroform and ethyl acetate extracts of leaves in which the percentage of anti-inflammatory effect continue to increase even after 5 hours. Also rhiofolin has strong anti- inflammatory activity. The results are listed in Table 2.

Antipyretic activity

All the tested extracts have antipyretic activity against yeast-induced hyperthermia. The effect reached its maximum nearly at the 3rd hour and continued till the 5th hour in all fractions except the n-hexane and n-butanol fractions of the stem and the total ethanolic extract of leaves.
The results are listed in Table 3.

Table 1: Antioxidant activity of the different extracts of *C. speciosa* A. St. Hil.

Extracts / Concentrations	Antioxidant %				
	1 mg	0.5 mg	0.25 mg	0.125 mg	0.0625mg
DPPH (Blank)	-	-	-	-	-
Quercetin (Reference)	100				
Leaves					
Total EtOH extract	78.7	76.7	65.6	63.7	41.6
n-Hexane fr.	76.1	74.8	59.8	46.4	42.02
Chloroform fr.	66.8	70.96	68.6	51.5	44.6
Ethyl acetate fr.	79.2	77.02	76.8	72.3	54.6
n-Butanol fr.	77.9	77.7	77.6	76.8	64.7
Stem					
Total EtOH extract	81.2	80	65.4	52.5	48.2
n-Hexane fr.	78.8	78.2	64.6	46.9	41.8
Chloroform fr.	72.5	58.9	52.7	42.3	37.9
Ethyl acetate fr.	72.5	69.9	69.2	67.5	65.5
n-Butanol fr.	63.3	58.3	44.5	39.7	42.5
Total EtOH extract of fruits	77.5	71.1	70.3	69.2	69
Rhiofolin	66.8	59.6	52.5	41.6	40.2

Table 2: Percentage of anti-inflammatory activity of the different extracts of *C. speciosa* A. St. Hil. carrageenan-induced hind paw edema model in rats.

Group	Dose mg/kg	% of inhibition				
		1 hr	2 hr	3 hr	4 hr	5 hr
Control	---	---	---	---	---	---
Indomethacin	8	26.79	34.16	41.42	41.1	42.23
Leaves						
Total eth. ext.	400	4.7	16.46	32.29	26.01	13.4
***n*-Hexane fr.**	400	6.09	13.44	20.57	16.69	4.56
$CHCl_3$ fr.	400	9.68	25.24	37.47	46.62	50.40
EtOAc fr.	400	6.64	27.98	42.92	43.31	53.60
***n* Butanol fr.**	400	7.19	24.96	32.7	32.14	9.39
Stem						
Total eth. ext.	400	9.54	28.53	45.10	32	23.48
***n*-Hexane fr.**	400	9.13	14.13	27	12.41	6.08
$CHCl_3$ fr.	400	6.78	14.27	26.02	18.21	10.08
EtOAc fr.	400	5.53	14.13	24	8.41	10.08
***n*-Butanol fr.**	400	9.27	14.54	36.38	26.34	14.5
Total eth. ext. of fruit	400	4.01	7.27	14.85	6.21	6.08
Rhiofolin	400	10.24	25.24	27.79	30.07	8.98

Data are expressed as mean ±S.E, n=6
Differences with respect to the control group were evaluated using the student t-test.
(**P*<0.05, ** *P*<0.01, *** P<0.001)

Table 3: Percentage of antipyretic activity of the different extracts of *C. speciosa* A. St. Hil. on yeast induced fever in rats.

Group	Dose mg/kg	Average rectal temperature (°C) ± S.E., n = 6				
		1 hr	2 hr	3 hr	4 hr	5hr
Control	---	38.53±0.05	38.57±0.10	38.64±0.06	38.37±0.09	38.47±0.04
Indomethacin	8	37.17±0.012***	36.10±0.015***	36.07±0.042***	36.07±0.012***	36.23±0.023***
Leaves						
Total eth. ext.	400	38.13±0.103*	37.8±0.089***	37.6±0.089***	37.76±0.052***	37.8±0.089*
n-Hexane fr.	400	38.1±0.089**	37.9±0.089**	37.63±0.052***	37.73±0.052***	37.8±0.103***
$CHCl_3$ fr.	400	38.03±0.052***	37.8±0.089***	37.53±0.052***	37.45±0.055***	37.45±0.054***
EtOAc fr.	400	38.1±0.089**	37.6±089***	37.43±0.103***	37.53±0.052***	37.43±0.052***
n-Butanol fr.	400	38.17±0.051***	37.57±0.103***	37.6±0.089***	37.57±0.051***	37.77±0.103***
Stem						
Total eth. ext.	400	38.17+0.136*	37.63+0.103***	37.53+0.051***	37.5+0.089***	37.53±0.051***
n-Hexane fr.	400	38.03±0.052***	37.77±0.103**	37.6±0.089***	37.63±0.136**	37.77±0.136**
$CHCl_3$ fr.	400	38.1±0.089**	37.67±0.103***	37.57±0.051***	37.77±0.103**	37.7±0.089***
EtOAc fr.	400	38.06±0.103**	37.63±0.103***	37.6±0.089***	37.8±0.089**	37.73±0.103***
n-Butanol fr.	400	38.13±0.136*	37.67±0.135**	37.57±0.103***	37.77±0.103**	37.9±0.089**
Total eth. ext. of fruist	400	38.23±0.136	37.8±0.089**	37.7±0.089***	37.7±0.089**	37.83±0.051***
Rhiofolin	400	38.4±0.09	37.8±0.08***	37.83±0.103**	37.67±0.103**	37.73±0.19**

Data are expressed as mean ±S.E, n=6
Differences with respect to the control group were evaluated using the student t-test. (*P<0.05, ** P<0.01, *** P<0.001)

Discussion

Chemical investigation of *Chorisia speciosa* A. St. Hil. leaves resulted in isolation and identification of ten compounds two of them are firstly reported in the family: *β*-amyrin, and Verbascoside, in addition to five compounds firstly isolated from the plant p-hydroxy benzoic acid, *β*- sitosterol-3-O-*β*-d-glucopyranoside, succinic acid, astragalin and cinaroside, in addition to stigmasterol, tiliroside and rhiofolin. The tested extracts and fractions showed significant antioxidant activity using DPPH assay.

The extracts have slightly higher antioxidant activity. The antioxidant activity is possibly due to presence of phenolic compounds as flavonoids. In addition they showed varying significant anti- inflammatory activity using carrageenan-induced edema model for inflammation. Carrageenan model of inflammation is said to be biphasic with the first phase attributed to the release of histamine, serotonin and kinins in the first hour, while the second phase is attributed to release of prostaglandins and lysosome enzymes [25]. The tested extracts inhibited both phases of inflammation.

The anti-inflammatory activity may be attributed to presence of flavonoids, sterols and triterpenes.

Conclusion

Ten compounds were isolated from the ethanolic leaves extract of *C. speciosa* A. St. Hil., including triterpenes, sterols and glycosides. Biological screening of different extracts for both antioxidant and anti- inflammatory activities revealed significant activity.

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