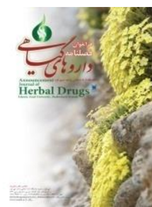
Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir



# Comparative polyphenol contents, free radical scavenging properties and effects on adipogenesis of *Chorisia Chodatii* and *Chorisia Speciosa*

John Refaat[1*], Samar Yehia Desoukey[1], Mahmoud Ahmed Ramadan[2], Mohamed Salah Kamel[1], Junkyu Han[3,4] and Hiroko Isoda[3,4]

[1]*Pharmacognosy Department, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt;*

[2]*Pharmacognosy Department, Faculty of Pharmacy, Assiut University, 71515 Assiut, Egypt;*

[3]*Alliance for Research on North Africa (ARENA), Tsukuba University, 305-8572 Tsukuba, Japan;*

[4]*Graduate School of Life and Environmental Sciences, Tsukuba University, 305-8572 Tsukuba, Japan;*

[*]**Email:** Johnrefaat82@yahoo.com

**ARTICLE INFO**

***Type:*** *Original Research*

***Topic:*** *Medicinal Plants*

***Received*** *November 27th 2014*

***Accepted*** *April 10th 2015*

***Key words:***

- ✓ *Adipogenesis*
- ✓ *Antioxidant*
- ✓ *Bombacaceae*
- ✓ Chorisia
- ✓ *Polyphenols*
- ✓ *3T3-L1 cells*

## ABSTRACT

**Background & Aim:** *Chorisia* is an important Bombacaceous plant that is traditionally used for a variety of ailments. Due to its richness in several bioactive phytocompounds, some *Chorisia* species showed a wide range of important biological effects. Accordingly, the present work was undertaken to comparatively evaluate the antioxidant potential and effects on adipogenesis of *Chorisia chodatii* Hassl. and *Chorisia speciosa* A. St.-Hil. in relation to their phenolic contents.

**Experimental:** Total polyphenol contents and free radical scavenging potentials of the total ethanol extracts of leaves, flowers, fruits and seeds of both species, in addition to four main fractions of their leaf and flower extracts, were evaluated using the Folin-Ciocalteu's method and the DPPH assay, respectively. Besides, their effects on adipogenesis were studied using the 3T3-L1 preadipocytes model. A phytochemical screening of their different phytoconstituents was also considered.

**Results & Discussion:** Total ethanol extracts along with their successive fractions of various parts of both species caused a concentration-dependent induction of 3T3-L1 preadipocytes differentiation, but with a noticeable reduction of the size of the lipid droplets at the lower concentrations 5 and 10 μg/ml. In addition, these extracts showed a strong evidence of their richness in free radical scavengers. The ethyl acetate, aqueous and chloroform fractions of different plant parts exhibited the greatest effects on adipogenesis, substantial free radical scavenging properties and the highest polyphenol contents, respectively.

**Industrial and practical recommendations:** Results collectively revealed that the observed effects of both *Chorisia* species on adipogenesis as well as their anti-radical properties are positively related to their pool of flavonoids and other phenolics. They also suggest their potential value in obesity-related disorders and for prevention of free radical mediated diseases. Further studies for investigating the molecular basis of their effects on adipogenesis accompanied by detailed phytochemical analysis, especially of their polar and flavonoids-rich extracts, will also be strongly recommended.

## 1. Introduction

Oxidative stress represents a common and threatening health theme that is associated with pathogenic mechanisms of many degenerative diseases including aging, arthritis, neurodegeneration, Alzheimer's, atherosclerosis, cancer and inflammatory disorders (Miller and Rice-Evans, 1997; Polterait, 1997). Free radicals produced on disturbance of normal redox state in biological systems can harmfully attack several biomolecules like proteins, lipids and DNA resulting in cell membrane damage due to lipid peroxidation, a decrease in membrane fluidity and DNA mutations leading to cancer (Pietta, 2000). Moreover, these reactive oxygen species activate the nuclear factor-κB, a nuclear transcription factor, resulting in an up-regulation of pro-inflammatory mediators such as interleukin-1, interleukin-8 and TNF-α. This in turn stimulates the immune response, increases oxidants' production and can lead to further tissue damage (Grimble, 1994). Potent scavengers of these free radical species may serve as a possible preventive intervention for free radical mediated diseases (Ames *et al.*, 1995). Since the well-known synthetic antioxidants; butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are reported to provoke some degree of carcinogenicity, current research efforts are devoted to evaluate the free radical scavenging potentials of medicinal plants as a logic strategy for development of new safe antioxidants of natural origin (Ito *et al.*, 1982).

On the other hand, obesity is another global health problem and a worldwide epidemic that is described as a chronic metabolic disorder caused by an imbalance between energy intake and expenditure (Roh and Jung, 2012). It represents a major risk factor for a number of health disorders including type-2 diabetes, hypertension, hyperlipidemia, osteoarthritis, asthma, cancer and cardiovascular diseases (Lee *et al.*, 2005). The World Health Organization estimates that by 2020, two-thirds of the global disease burden will be attributable to chronic diseases associated with obesity (Chopra *et al.*, 2002). Obesity is produced by an increase in adipose tissue mass which results from the multiplication of fat cells through adipogenesis and the increased deposition of cytoplasmic triglycerides (Lefterova and Lazar, 2009). Physiologically, adipogenesis is described as the process by which undifferentiated preadipocytes are converted to fully differentiated adipocytes (Otto and Lane, 2005). That's why; the size of adipose tissue mass is a function of both adipocyte number and size. An increase in adipose tissue mass can result from hyperplastic growth which occurs primarily by mitotic activity in precursor cells or by hypertrophic growth which occurs mainly by lipid accumulation within the cell (Johnson and Greenwood, 1988). Development of obesity is also regulated by genetic, endocrine, metabolic, neurological, pharmacological, environmental and nutritional factors (Farmer and Auwerx, 2004). 3T3-L1 preadipocytes cell line is one of the most reliable models to study the events during conversion of preadipocytes into mature adipocytes. During terminal differentiation, the fibroblast-like preadipocytes undergo a series of morphological and biochemical changes to eventually accumulate lipid droplets. Besides, the *in vitro* differentiation of fat cells recapitulates most of the characteristic features of *in vivo* adipogenesis including morphological change, growth arrest, high expression of lipogenic genes and production of hormones like leptin, resistin and TNF-α (Kirkland *et al.*, 1990). As a sleeping giant for drug development, a variety of natural products including crude extracts and isolated phytocompounds from plants can induce body weight reduction and prevent diet-induced obesity by different mechanisms, therefore, they have been widely tested for their anti-obesity potentials (Yun, 2010).

*Chorisia* (Syn. *Ceiba* (Ravenna, 1998)) is a genus of deciduous trees belongs to family Bombacaceae and comprises about twenty species. They are commonly known as silk floss, drunken or bottle trees (Bailey, 1976; Huxley, 1992). These plants are traditionally used for many disorders e.g. headache, fever, diabetes, diarrhoea, parasitic infections, hypertension, mental troubles, peptic ulcer, rheumatism and asthma (Adjanohoun, 1988; Ngounou *et al.*, 2000). A few number of *Chorisia* species were subjected to phytochemical analyses that yielded a number of flavonoids, anthocyanins, sterols, triterpenes and carbohydrates (Refaat *et al.*, 2013). It was also reported that some *Chorisia* species showed a wide range of useful anti-inflammatory, hepatoprotective, cytotoxic, antioxidant, hypoglycaemic and antimicrobial effects with high safety margins and $LD_{50}$ up to 7-8 g/kg in lab animals (Hafez *et al.*, 2003; Hassan, 2009; El-Alfy *et al.*, 2010; Ashmawy *et al.*, 2012). Consequently, this

study was designed to comparatively investigate, for the first time, the effects of different extracts and fractions of various plant parts of *Chorisia chodatii* Hassl. (*CC*) and *Chorisia speciosa* A. St.-Hil. (*CS*) on lipogenesis during differentiation of 3T3-L1 cells and their antioxidant properties in relation to their total polyphenol contents. In addition, a preliminary qualitative analysis of their phytochemicals was also considered.

## 2. Materials and Methods

### *2.1. Plant Material*

Leaves, flowers, fruits and seeds of both *CC* and *CS* were collected from plants cultivated in the campus of Minia University, Minia, Egypt. Flowers of both species were collected in October 2009; leaves were collected before the next flowering stage in July 2010, whereas their fruits and seeds were collected in April 2011. Botanical authentication of both plants was confirmed by Prof. Ahmed Abdel-Monem, Department of Horticulture, Faculty of Agriculture, Minia University, Minia, Egypt. Voucher specimens [Mn-Ph-Cog-001 (for *CC*) and Mn-Ph-Cog-002 (for *CS*)] were deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University.

### *2.2. Chemicals and Reagents*

Different chemicals and solvents used for extraction, fractionation and phytochemical screening procedures were purchased from El-Nasr Company for Pharmaceuticals and Chemicals, A.R.E. Dulbecco's modified Eagle's medium (DMEM) (no. RNBC 4111) and 0.25% Trypsin-EDTA solution (no. SLBC1657) were obtained from Sigma Chemical Co, St. Louis, MO, USA. Adipogenesis assay kit (no. 10006908) was obtained from Cayman Chemicals (Ann. Arbor, MI, USA). Penicillin (5000 units/ml) / streptomycin (5000 µg/ml) (PS) (no. 17-603E) was obtained from Lonza Walkersville, MD, USA. Trypan blue (no. 1525-061) and fetal bovine serum (FBS) (no. 10437-028) were obtained from Gibko, USA. DMSO (no. 046-21981), ethanol (for samples dilution and preparation of reagents, no. 057-00456), gallic acid monohydrate (no. 070-00122), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (no. ALJ 4179), and Folin-Ciocalteu reagent (no. 279-08895) were purchased from Wako Pure Chemical Industries, Japan. Sodium carbonate (no. 28-2170-5) was obtained from Sigma-Aldrich, USA. Trolox (no. 440-1102-752) was purchased from Calbiochem, Denmark. Morpholinoethanesulfonic acid monohydrate (MES) (no. 349-01623) was obtained from Dojindo, Japan.

### *2.3. Preparation of total extracts and different fractions*

The air-dried organs of both species were reduced to coarse powders and macerated separately in ethanol (70%). The ethanol solutions were then concentrated independently under reduced pressure at 40°C to a syrupy consistency. The solvent-free residues of total extracts of *CC* flowers and leaves, in addition to that of *CS* flowers were separately suspended in distilled water and subjected to exhaustive fractionation steps using light petroleum ether, chloroform and ethyl acetate, successively. The organic layer in each step was collected and concentrated independently to give the corresponding fraction, whereas the aqueous fractions were obtained by concentration of the remaining mother liquor for each plant part (Table 1). Each total extract and fraction (10 mg) was separately dissolved in 500 µl DMSO to prepare a stock solution of 20 mg/ml and stored at 4°C.

### *2.4. Phytochemical screening*

Different extracts and fractions of *CC* and *CS* were tested for presence of carbohydrates and/or glycosides, alkaloids and/or nitrogenous compounds, flavonoids, anthocyanins, quinones, coumarins, steroids, triterpenoids, saponins, cardenolides, tannins and proteins. Phytochemical screening was performed using chemical methods and thin layer chromatography according to the standard procedures (Trease and Evans, 1989; Wagner and Bladht, 1996).

### *2.5. Determination of total polyphenol content*

Total phenolic contents were determined using Folin-Ciocalteu's reagent according to the method described by Lister and Wilson (2001). Five concentrations of each sample (50, 100, 150, 250 and 500 µg/ml) were prepared by suitable dilution of the initially prepared stock solutions with ethanol. Gallic acid (at 0, 60, 180 and 300 µM) was used as a standard, and results were expressed in terms of gallic acid equivalence (GAE) in µM. Solutions of various gallic acid concentrations (50 µl/well) and those of the tested samples (25 µl/well, diluted with an equal volume of

Milli-Q water) were oxidized with 50 µl/well of Folin-Ciocalteu's reagent [diluted with Milli-Q water (1:1)]. After incubation for 3 min at room temperature, 50 µl of 10% aqueous sodium carbonate solution were added to all wells. The 96-well plates were allowed to stand in the dark at room temperature for 30 min and the absorbances were then read at 750 nm using a microplate reader (Dainippon Pharmaceutical, Osaka, Japan). Analyses were done in three replicates.

### *2.6. DPPH free radical scavenging assay*

The free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Blois (1958). Sample stock solutions were diluted to final concentrations of 50, 100, 150, 250 and 500 µg/ml in ethanol. Trolox (at 600, 800, 1000, 1200 and 1400 µM) was used as a positive standard and results were expressed in terms of trolox equivalence in µM. Two working solutions (WS) containing MES, ethanol and Milli-Q water either with DPPH (for WS+) or without (for WS-) were prepared. For each 96-well plate, a set of wells containing 10 µl/well of each trolox concentration or the tested sample was treated with 190 µl/well of (WS+), whereas another equivalent set containing 10 µl/well of each trolox concentration or the tested sample was treated with 190 µl/well of (WS-). A negative control was represented by a mixture of (WS-, 190 µl/well) and ethanol (10 µl/well). The plates were incubated in dark at room temperature for 3 min and the absorbances were then read at 520 nm using a microplate reader (Dainippon Pharmaceutical, Osaka, Japan). Tests were carried out in triplicates.

### *2.7. Cell Culture*

3T3-L1 preadipocytes (Riken Cell Bank, Ibaraki, Japan) were grown to confluence in maintenance medium comprised of DMEM supplemented with 10% FBS and 5% penicillin/streptomycin and incubated at 37°C in 5% $CO_2$.

### *2.8. Adipogenesis Assay*

Based on the standard protocol (Erding *et al.,* 1996), an equal concentration of 3T3-L1 cells (3 x $10^5$ cells/ml) was used for seeding of 96-well plates. Two days post confluence (Day 0), preadipocyte differentiation was initiated using adipogenesis assay kit by changing the maintenance medium to induction medium containing the adipogenic cocktail; 3-isobuthyl-1-methylxanthine (IBMX), dexamethasone and insulin (10 µl of each in 10 ml DMEM supplemented with 10% FBS for each plate). This induction medium was used for differentiation of the control group (positive control), whereas the same medium containing the tested sample was used for the treatment groups. On day 3, the induction medium was replaced with insulin medium (10 µl insulin in 10 ml DMEM containing 10% FBS for each plate) for the control group or insulin medium containing the tested sample for the treatment groups. Five days after induction (Day 5), the medium was replaced with fresh insulin medium (for the control group) or insulin medium containing the tested sample (for the treatment groups). The accumulation of lipid droplets was monitored by day 7 under the microscope. A group of undifferentiated preadipocytes served as the negative control throughout the various steps of the test. Four concentrations (5, 10, 50 and 100 µg/ml) of each sample were prepared by suitable dilution of the initial stock solutions with the induction or insulin medium according to each step. Cells were incubated at 37°C with 5% $CO_2$ throughout different stages of the experiment.

### *2.9. Staining of lipid droplets and quantification*

After accumulation of lipid droplets, the differentiated cells were fixed with diluted lipid droplets assay fixative (75 µl/well) and incubated for 15 min at room temperature. The fixative was then washed out with a wash solution (two times for 5 min, 100 µl/well). After complete drying, cells were stained with Oil Red O dye (75 µl/well) for 20 min at room temperature. The unbound stain was washed out several times with distilled water and again with a wash solution (two times for 5 min, 100 µl/well). Microscopic images of fat accumulation were then captured at this point. After complete drying, the bound dye was eluted using dye extraction solution (100 µl/well) with gentle shaking for 15-30 min. The absorbances were then read at 490 nm using a microplate reader (Dainippon Pharmaceutical, Osaka, Japan).

### *2.10. Statistical Analysis*

Results were expressed as mean ± standard deviation. The significance of data was evaluated using Student's *t*-test for comparison between two groups. The values of $p < 0.05$ and $p < 0.01$ versus control were considered statistically significant.

## 3. Results and discussion

### 3.1. Final yields of total extracts and fractions

Weights of dried powders (g) of various parts of *CC* and *CS* together with the obtained yields for their total ethanol extracts and successive fractions (g) are depicted in Table 1.

### 3.2. Phytochemical Screening

Qualitative phytochemical analysis of the total ethanol extracts and their successive fractions of different parts of *CC* and *CS* revealed their richness in flavonoids, anthocyanins, steroids, triterpenoids, tannins, carbohydrates and glycosides, whereas quinones, saponins, cardenolides, alkaloids and nitrogenous substances were totally absent in all samples as summarized in Table 2.

### 3.3. Total polyphenol contents (TPC) and DPPH scavenging activity

Recent studies showed that the anti-radical capacity of most plant products is usually associated with their phenolic substances. Plant polyphenols are a large group of phytochemicals including phenolic acids, flavonoids, carotenoids, tannins, tocopherols, glutathione, ascorbic acid and antioxidant enzymes. Natural antioxidants have been extensively studied for decades in order to find compounds protecting against free radical-induced diseases (Kaur and Arora, 2009; Newman and Cragg, 2007). That's why and based on our phytochemical screening results, the TPC and anti-radical activity of total ethanol extracts and successive fractions of four organs of *CC and CS* were comparatively investigated for the first time. TPC were determined spectrophotometrically using the Folin–Ciocalteu's method which is a colorimetric oxidation/reduction assay for phenolic compounds based on oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (Folin–Ciocalteu's reagent). The absorption intensity of the resulting blue products at 750 nm is proportional to the concentration of phenols (Slinkard and Singleton, 1977). TPC were calculated using the standard regression curve and expressed as GAE in µM. The results displayed that the ethyl acetate, aqueous and chloroform fractions of different plant parts possess the highest contents, respectively, whereas the petroleum ether extracts were the poorest ones (Tables 3-5; for graphical presentation see Additional File 1). Additionally, total ethanol extracts of seeds of both species showed less TPC compared to those of other organs (Table 3).

On the other hand, the DPPH assay was used to evaluate the antioxidant potential of both plants. This test is widely used because DPPH radicals are much more stable and easier to handle than oxygen free radicals. Because of its odd electron, DPPH free radicals give a strong absorption band at 517-520 nm in visible spectroscopy (deep violet colour) (Tominaga *et al.*, 2005). The scavenging effects of different extracts were expressed as the equivalent concentration to trolox in µM using the standard regression curve. Different extracts and fractions of both species, except petroleum ether fractions, showed concentration-dependent scavenging abilities by quenching DPPH radicals (Tables 6-8; for graphical presentation see Additional File 2). The ethyl acetate, aqueous and chloroform partitions exhibited the highest activities, respectively. Besides, total ethanol extracts of seeds of both species showed the least scavenging properties among the tested total extracts of other plant parts (Table 6).

**Table 1.** Final yields (g) of total extracts and fractions of different organs of *CC* and *CS*.

| **Species** | ***C. chodatii*** | | | | ***C. speciosa*** | | | |
|---|---|---|---|---|---|---|---|---|
| **Plant Part** | **Flowers** | **Leaves** | **Fruits** | **Seeds** | **Flowers** | **Leaves** | **Fruits** | **Seeds** |
| Dried powder (g) | 100 | 100 | 10 | 10 | 100 | 100 | 10 | 10 |
| Total ethanol extract | 12.52 | 7.96 | 1.59 | 0.39 | 13.41 | 7.25 | 1.22 | 0.30 |
| Petroleum ether fraction | 2.24 | 3.11 | - | - | 2.51 | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Chloroform fraction | 0.23 | 0.21 | - | - | 0.29 | - | - | - |
| Ethyl acetate fraction | 0.78 | 0.37 | - | - | 0.83 | - | - | - |
| Aqueous fraction | 8.51 | 3.92 | - | - | 8.93 | - | - | - |

**Table 2.** Qualitative analysis of phytochemicals in total extracts and fractions of *CC* and *CS* *.

| **Species** | ***C. chodatii*** | | | | ***C. speciosa*** | | | |
|---|---|---|---|---|---|---|---|---|
| **Extract** | **Flowers** | **Leaves** | **Fruits** | **Seeds** | **Flowers** | **Leaves** | **Fruits** | **Seeds** |
| Total ethanol extract | F, A, St, Tr, T, Co, G, C, Pr | F, A, St, Tr, T, Co, G, C | F, A, St, Tr, T, Co, G, C, Pr | F, A, St, Tr, T, Co, G, C, Pr | F, A, St, Tr, T, Co, G, C, Pr | F, A, St, Tr, T, Co, G, C | F, A, St, Tr, T, Co, G, C, Pr | F, A, St, Tr, T, Co, G, C, Pr |
| Pet. ether fraction | St, Tr | St, Tr | NT | NT | St, Tr | NT | NT | NT |
| $CHCl_3$ fraction | F, Co, St | F, Co, St | NT | NT | F, Co, St | NT | NT | NT |
| EtOAc fraction | F, Co, G | F, Co, G | NT | NT | F, Co, G | NT | NT | NT |
| Aqueous fraction | F, A, T, G, C, Pr | F, A, T, G, C | NT | NT | F, A, T, G, C, Pr | NT | NT | NT |

* Phytoconstituents with positive results in different screening tests were only recorded for each sample. F: flavonoids, A: anthocyanins, St: steroids, Tr: triterpenoids, T: tannins, Co: coumarins, G: glycosides, C: carbohydrates, Pr: proteins, NT: not tested.

**Table 3.** Total polyphenol contents of total ethanol extracts of different parts of *CC* and *CS*.

| | **GAE in µM (mean ± SD)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Sample concentration (µg/ml)** | | | | | | | | | |
| **Plant part** | ***C. chodatii*** | | | | | ***C. speciosa*** | | | | |
| | **50** | **100** | **150** | **250** | **500** | **50** | **100** | **150** | **250** | **500** |
| Flowers | 30.36 ± 4.177 | 52.01 ± 2.928 | 65.36 ± 4.231 | 86.10 ± 4.988 | 120.2 ± 4.344 | 31.70± 2.815 | 58.76 ± 5.123 | 74.82 ± 2.995 | 104.9 ± 3.983 | 127.81 ± 3.24 |
| Leaves | 21.51 ± 2.217 | 35.45 ± 2.566 | 52.95 ± 1.673 | 71.01 ± 5.697 | 103.6 ± 3.955 | 16.38± 4.399 | 42.57 ± 4.045 | 71.93 ± 3.252 | 86.18 ± 1.473 | 110.12 ± 1.505 |
| Fruits | 21.07 ± 1.615 | 36.33 ± 4.058 | 65.47 ± 3.679 | 78.66 ± 2.330 | 109.8 ± 5.076 | 24.12± 1.798 | 52.01 ± 2.964 | 66.33 ± 5.629 | 87.65 ± 3.192 | 115.7 ± 4.134 |
| Seeds | 11.20 ± 1.263 | 21.32 ± 1.082 | 25.16 ± 3.286 | 54.48 ± 3.156 | 87.83 ± 3.855 | 13.66 ± 1.774 | 22.67 ± 1.723 | 30.64 ± 3.923 | 61.46 ± 3.101 | 87.75 ± 4.895 |

**Table 4.** Total polyphenol contents of different fractions of *CC* and *CS* flowers.

| | **Equivalent concentration to gallic acid in µM (mean ± SD)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Sample concentration (µg/ml)** | | | | | | | | | |
| **Sample** | ***C. chodatii*** | | | | | ***C. speciosa*** | | | | |
| | **50** | **100** | **150** | **250** | **500** | **50** | **100** | **150** | **250** | **500** |
| Total ethanol extract | 30.36 ± 4.177 | 52.01 ± 2.928 | 65.36 ± 4.231 | 86.10 ± 4.988 | 120.3 ± 4.344 | 31.70 ± 2.815 | 58.76 ± 5.123 | 74.82 ± 2.995 | 104.9 ± 3.983 | 127.8 ± 3.24 |
| Pet. ether fraction | 9.099 ± 1.525 | 11.51 ± 1.254 | 18.57 ± 0.874 | 31.51 ± 1.543 | 59.51 ± 2.430 | 6.41 ± 1.859 | 11.74 ± 1.87 | 16.57 ± 3.821 | 32.69 ± 3.46 | 65.01 ± 3.799 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Chloroform fraction | 34.9 ± 3.980 | 71.36 ± 1.286 | 103.2 ± 4.677 | 146.1 ± 4.503 | 217.3 ± 4.416 | 36.75 ± 1.812 | 80.78 ± 3.477 | 120.1 ± 2.316 | 154.8 ± 3.6 | 229.7 ± 2.686 |
| Ethyl acetate fraction | 88.94 ± 4.287 | 142.0 ± 2.937 | 165.9 ± 4.404 | 225.5 ± 5.058 | 267.3 ± 2.811 | 109.5 ± 0.795 | 158.9 ± 5.683 | 178.7 ± 3.293 | 232.8 ± 2.628 | 285.2 ± 4.092 |
| Aqueous fraction | 65.05 ± 6.235 | 123.3 ± 1.673 | 161.1 ± 3.879 | 205.4 ± 3.971 | 246.1 ± 2.681 | 73.29 ± 4.296 | 128.3 ± 1.238 | 154.4 ± 6.375 | 200.7 ± 5.041 | 255.5 ± 4.267 |

**Equivalent concentration to trolox in µM (mean ± SD)**

| Sample | Equivalent concentration to gallic acid in µM (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Sample concentration (µg/ml) | | | | |
| | **50** | **100** | **150** | **250** | **500** |
| Total ethanol extract | 21.51 ± 2.217 | 35.45 ± 2.566 | 52.95 ± 1.673 | 71.01 ± 5.697 | 103.69 ± 3.955 |
| Pet. ether fraction | 6.51 ± 1.81 | 11.48 ± 1.578 | 19.4 ± 1.854 | 33.63 ± 1.66 | 50.81 ± 2.163 |
| Chloroform fraction | 29.68 ± 3.299 | 63.33 ± 6.41 | 84.13 ± 5.332 | 131.25 ± 2.363 | 209.17 ± 5.256 |
| Ethyl acetate fraction | 80.82 ± 5.302 | 121.81 ± 7.111 | 153.33 ± 4.404 | 216.76 ± 1.693 | 252.62 ± 3.334 |
| Aqueous fraction | 56.85 ± 1.956 | 105.21 ± 5.255 | 137.42 ± 6.378 | 188.47 ± 2.253 | 233.59 ± 3.464 |

**Table 5.** Total polyphenol contents of different fractions of *CC* leaves.

**Table 6.** DPPH scavenging activity of total ethanol extracts of different parts of *CC* and *CS*.

| Plant part | Equivalent concentration to trolox in µM (mean ± SD) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample concentration (µg/ml) | | | | | | | | | |
| | *C. chodatii* | | | | | *C. speciosa* | | | | |
| | **50** | **100** | **150** | **250** | **500** | **50** | **100** | **150** | **250** | **500** |
| Flowers | 31.63 ± 2.027 | 69.71 ± 3.879 | 92.6 ± 4.132 | 122.6 ± 2.998 | 242.8± 2.258 | 43.33± 1.225 | 75.13± 4.636 | 99.35± 6.425 | 131.8± 1.571 | 248.7±4.699 |
| Leaves | 31.46 ± 2.262 | 53.3 ± 2.215 | 74.6 ± 3.956 | 104.8 ± 1.916 | 211.9± 4.764 | 31.8± 1.319 | 64.46± 5.639 | 78.91± 2.502 | 108.6± 1.533 | 219.2± 2.324 |
| Fruits | 35.71 ± 2.769 | 59.33 ± 1.532 | 84.88 ± 4.693 | 116.5 ± 6.004 | 226.2± 3.615 | 37.37± 3.123 | 67.95± 5.480 | 90.44± 8.462 | 125.2± 2.945 | 226.8± 4.735 |
| Seeds | 13.39 ± 2.085 | 24.72 ± 3.028 | 47.51 ± 5.217 | 78.84 ± 3.304 | 133.5± 3.275 | 21.14± 2.274 | 29.47± 4.796 | 57.05± 3.099 | 76.06± 2.419 | 125.6± 2.836 |

An important feature that can be observed is that, different extracts of *CC* showed similar polyphenol contents to their corresponding ones of *CS*, with those of the latter were relatively higher. Furthermore, based on the same tendency observed in the results of DPPH and TPC tests, it could be inferred that the phenolic principles are strongly contributing to the scavenging properties of these samples. Recent studies demonstrated that the interaction of a potential antioxidant with DPPH depends on its structural conformation. The number of DPPH molecules that are reduced seems to be correlated with the number of available hydroxyl groups (Brand-Williams *et al.*, 1995). It is also strongly suggested that the DPPH free radical abstracts the phenolic hydrogen of the electron-donating molecule and this could be the general mechanism of the scavenging action of flavonoids, for example (Ratty *et al.*, 1995). Subsequently, among all fractions, the more polar ones (ethyl acetate and aqueous) were the strongest DPPH scavengers and

exhibited their effects in a concentration-dependent manner. The good activity of these polar extracts is probably due to the presence of substances with available hydroxyl groups as a structural requirement e.g. flavones and flavonols.

**Table 7.** DPPH scavenging activity of different fractions of *CC* and *CS* flowers**.**

| Sample | Sample concentration (μg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *C. chodatii* | | | | | *C. speciosa* | | | | |
| | 50 | 100 | 150 | 250 | 500 | 50 | 100 | 150 | 250 | 500 |
| Total ethanol extract | 31.63 ± 2.027 | 69.71 ± 3.879 | 92.6 ± 4.132 | 122.6 ± 2.998 | 242.8 ± 2.258 | 43.33 ± 1.225 | 75.13 ± 4.636 | 99.35 ± 6.425 | 131.8 ± 1.571 | 248.7 ± 4.699 |
| Pet. ether fraction | 4.4 ± 1.225 | 12.06 ± 1.696 | 19.88 ± 1.815 | 38.96 ± 5.138 | 59.05 ± 4.077 | 5.06 ± 1.225 | 12.06 ± 2.639 | 28.06 ± 3.959 | 41.76 ± 3.231 | 77.8 ± 3.582 |
| Chloroform fraction | 75.21 ± 3.241 | 184.1 ± 3.605 | 228.5 ± 3.091 | 272.4 ± 6.248 | 472.6 ± 4.254 | 76.39 ± 6.608 | 191.8 ± 7.252 | 245.2 ± 2.903 | 309.3 ± 6.896 | 490.7 ± 5.184 |
| Ethyl acetate fraction | 116.8 ± 2.410 | 254.0 ± 5.480 | 336.0 ± 6.398 | 434.1 ± 5.923 | 827.6 ± 5.073 | 133.6 ± 2.298 | 274.8 ± 5.219 | 343.7 ± 4.419 | 504.2 ± 2.902 | 841.4 ± 5.993 |
| Aqueous fraction | 91.76 ± 6.283 | 224.4 ± 3.716 | 315.9 ± 4.302 | 398.5 ± 2.904 | 805.1 ± 5.879 | 110.7 ± 7.701 | 248.8 ± 6.376 | 324.8 ± 5.208 | 413.9 ± 2.522 | 808.3 ± 2.716 |

**Table 8.** DPPH scavenging activity of different fractions of *CC* leaves.

| Sample | Equivalent concentration to trolox in μM (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Sample concentration (μg/ml) | | | | |
| | 50 | 100 | 150 | 250 | 500 |
| Total ethanol extract | 31.46 ± 2.262 | 53.3 ± 2.215 | 74.6 ± 3.956 | 104.8 ± 1.916 | 211.9 ± 4.764 |
| Petroleum ether fraction | 7.16 ± 1.543 | 11.54 ± 2.415 | 16.49 ± 5.222 | 40.01 ± 8.000 | 59.27 ± 5.148 |
| Chloroform fraction | 58.81 ± 5.244 | 165.49 ± 5.290 | 221.9 ± 4.855 | 256.83 ± 2.702 | 466.22 ± 6.416 |
| Ethyl acetate fraction | 108.12 ± 2.341 | 229.09 ± 4.849 | 317.40 ± 7.502 | 427.83 ± 6.036 | 816.79 ± 5.383 |
| Aqueous fraction | 83.32 ± 6.312 | 207.16 ± 8.378 | 286.11 ± 3.098 | 385.66 ± 7.169 | 781.94 ± 3.444 |

**Table 9.** Effect of total ethanol extracts of different parts of *CC* and *CS* on adipogenesis in 3T3-L1 cells.

| Plant part | Relative value of Oil Red O (% of control) (mean ± SD) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sample concentration (μg/ml) | | | | | | | |
| | *C. chodatii* | | | | *C. speciosa* | | | |
| | 5 | 10 | 50 | 100 | 5 | 10 | 50 | 100 |
| Flowers | 98.38 ± 4.483 | 100.7 ± 4.476 | 107.6 ± 4.126* | 115.06 ± 2.403** | 93.52 ± 4.397 | 101.4 ± 5.004 | 109.78 ± 3.519** | 113.65 ± 4.25** |
| Leaves | 90.78 ± 7.768 | 101.09 ± 2.0296 | 112.49 ± 5.854** | 114.52 ± 6.80** | 101.17 ± 5.55 | 103.27 ± 6.365 | 111.36 ± 7.337* | 119.03 ± 6.306** |
| Fruits | 92.92 ± 4.779 | 102.84 ± 2.148 | 112.01 ± 3.873** | 114.85 ± 3.268** | 98.33 ± 6.602 | 108.86 ± 3.179* | 116.06 ± 5.807** | 120.56 ± 4.081** |
| Seeds | 94.66 ± 4.864 | 98.94 ± 5.023 | 115.5 ± 5.133** | 119.92 ± 1.866** | 93.22 ± 5.807 | 97.6 ± 7.454 | 108.11 ± 2.517* | 119.81 ± 4.001** |

* Values are significant at $P < 0.05$ versus control ** Values are significant at $P < 0.01$ versus control

**Table 10.** Effect of different fractions of *CC* and *CS* flowers on adipogenesis in 3T3-L1 cells.

| Sample | Relative value of Oil Red O (% of control) (mean ± SD) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sample concentration (µg/ml) | | | | | | | |
| | *C. chodatii* | | | | *C. speciosa* | | | |
| | 5 | 10 | 50 | 100 | 5 | 10 | 50 | 100 |
| Total ethanol extract | 98.38 ± 4.483 | 100.7 ± 4.476 | 107.6 ± 4.126* | 115.0 ± 2.403** | 93.52 ± 4.397 | 101.4 ± 5.004 | 109.7 ± 3.519** | 113.6 ± 4.25** |
| Petroleum ether fraction | 93.89 ± 3.426 | 100.9 ± 5.091 | 104.7 ± 5.731 | 108.2 ± 5.186* | 104.8 ± 4.743 | 108.3 ± 4.982 | 111.5 ± 6.211* | 113.0 ± 3.491** |
| Chloroform fraction | 102.1 ± 2.910 | 105.5 ± 3.634 | 113.5 ± 1.784** | 118.5 ± 2.176** | 113.2 ± 7.565* | 117.3 ± 6.738** | 128.1 ± 5.964** | 135.6 ± 6.04** |
| Ethyl acetate fraction | 107.3 ± 3.051 | 118.4 ± 6.973** | 139.1 ± 3.776** | 156.9 ± 6.508** | 108.9 ± 5.343 | 115.7 ± 5.231* | 156.4 ± 7.512** | 164.9 ± 4.954** |
| Aqueous fraction | 102.4 ± 4.209 | 114.7 ± 9.067* | 127.0 ± 2.011** | 136.8 ± 6.991** | 110.5 ± 5.901 | 115.7 ± 7.693* | 132.8 ± 3.717** | 149.3 ± 5.594** |

* Values are significant at $P < 0.05$ versus control ** Values are significant at $P < 0.01$ versus control

**Table 11.** Effect of different fractions of *CC* leaves on adipogenesis in 3T3-L1 cells.

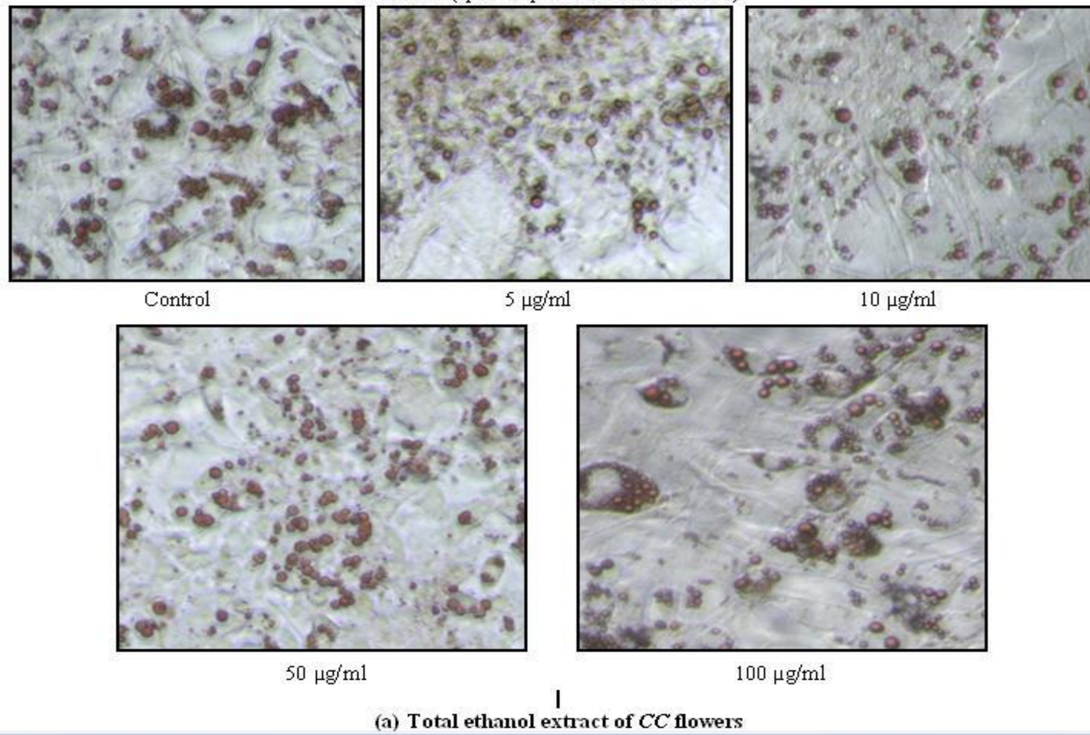
| Sample | Relative value of Oil Red O (% of control) (mean ± SD) | | | |
|---|---|---|---|---|
| | Sample concentration (µg/ml) | | | |
| | 5 | 10 | 50 | 100 |
| Total ethanol extract | 90.78 ± 7.768 | 101.09 ± 2.0296 | 112.49 ± 5.854** | 114.52 ± 6.80** |
| Petroleum ether fraction | 95.31 ± 6.041 | 105.18 ± 2.136 | 108.31 ± 2.006* | 111.93 ± 4.184** |
| Chloroform fraction | 107.91 ± 3.228 | 111.39 ± 7.278* | 119.16 ± 3.401** | 129.89 ± 5.474** |
| Ethyl acetate fraction | 110.96 ± 8.498 | 112.55 ± 7.181* | 125.89 ± 5.273** | 150.46 ± 2.566** |
| Aqueous fraction | 101.45 ± 5.899 | 113.61 ± 8.498* | 131.51 ± 4.936** | 135.91 ± 2.509** |

* Values are significant at $P < 0.05$ versus control ** Values are significant at $P < 0.01$ versus control

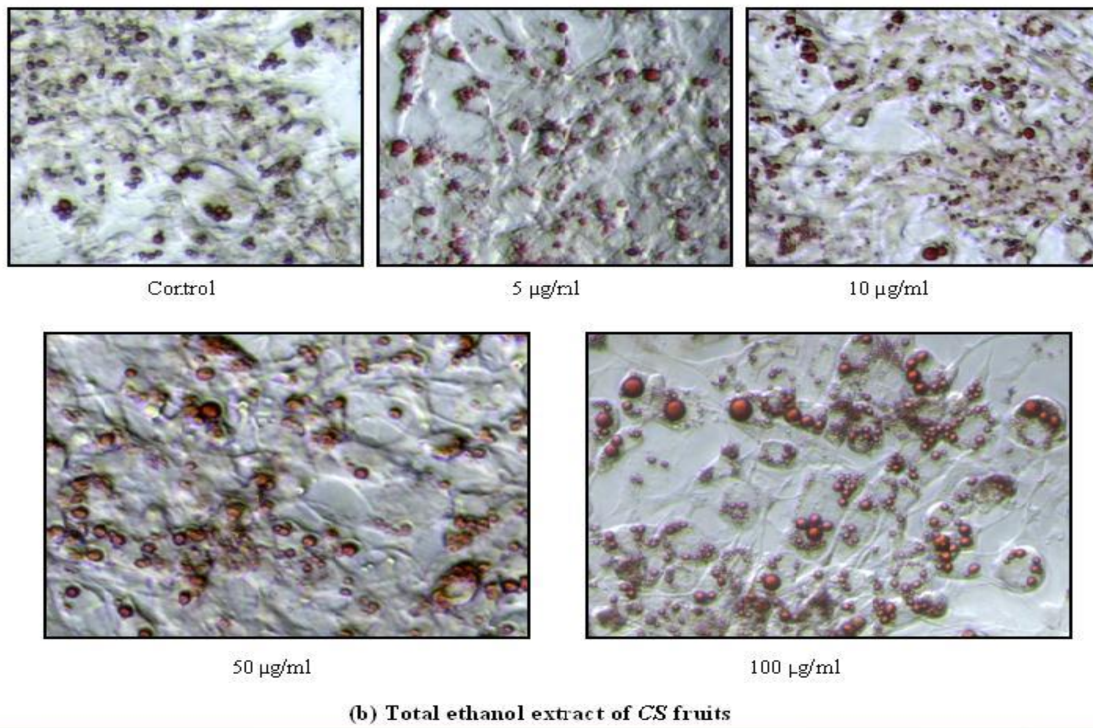
### *3.4. Adipogenesis Assay*

In this assay, twenty samples representing total ethanol extracts and fractions of various parts of *CC* and *CS* were screened for their influence on lipogenesis in 3T3-L1 adipocytes at 5, 10, 50 and 100 µg/ml. Theirs effects on fat accumulation were evaluated through the stained lipid droplets and expressed in the relative values of Oil Red O as percentages of the positive control group (Tables 9-11; for graphical presentation see Additional File 3). In comparison with the differentiated control cells, all samples did not significantly affect the number of the formed lipid droplets at 5 µg/ml but a reduction in their sizes was observed for most samples (Figure 1). At 10 µg/ml, the total ethanol extracts of different plant parts of both species increased formation of lipid droplets but the effect was statistically insignificant except for *CS* fruits (Table 9). Conversely, the chloroform, ethyl acetate and aqueous fractions of *CC* flowers and leaves, in addition to those of *CS* flowers significantly enhanced the formation of fat droplets at 10 µg/ml. On the other hand, a marked and significant induction of adipocyte maturation and fat accumulation was observed for most samples at the higher concentrations 50 and 100 µg/ml (Tables 10 and 11). At these concentrations, different samples provoked an increase in both the number and size of the formed lipid droplets and consequently, the amount of bound Oil Red O dye increased. Among all the tested fractions, the ethyl acetate, aqueous and chloroform partitions exhibited the largest stimulatory effects on adipogenesis at the various concentrations, respectively, whereas the petroleum ether fractions showed significant lipogenic effects only at 50 and 100 µg/ml (Tables 10 and 11).

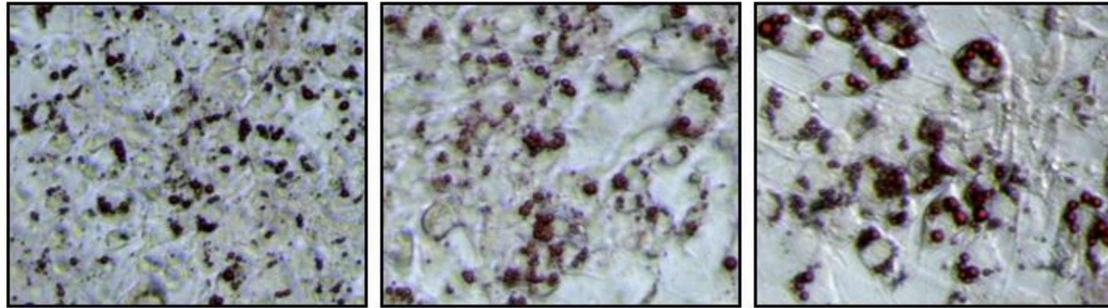
**Figure 1.** Selected microscopic images showing induction of adipocyte differentiation by *CC* and *CS* (lipid droplets are stained in red)



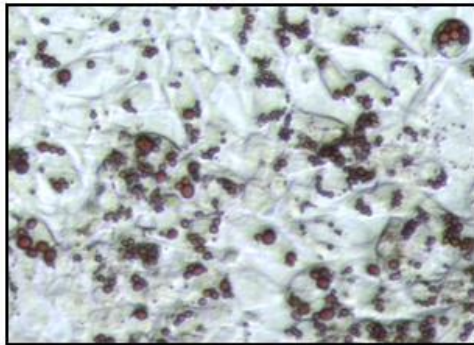

**(a) Total ethanol extract of *CC* flowers**



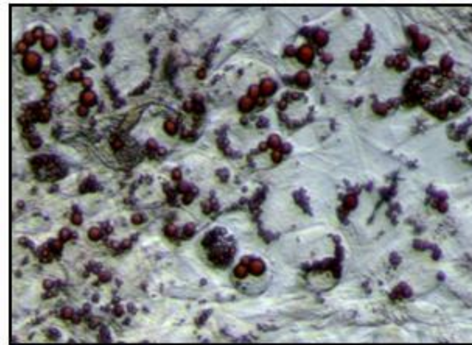

**(b) Total ethanol extract of *CS* fruits**



Control 5 µg/ml 10 µg/ml

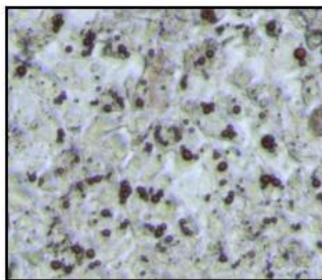


50 µg/ml

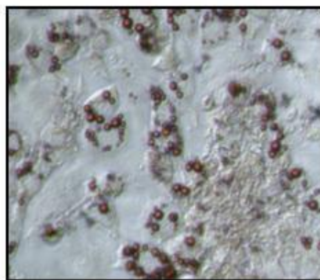


100 µg/ml

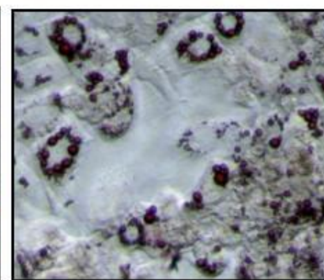
**(c) Ethyl acetate fraction of *CS* flowers**



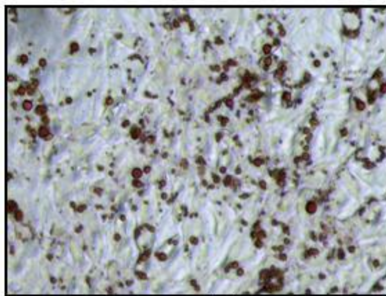
Control



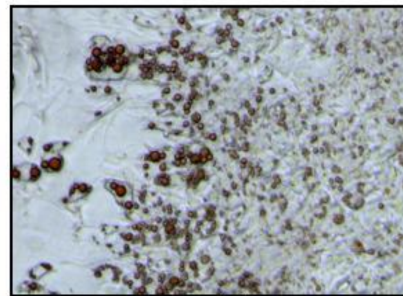
5 µg/ml



10 µg/ml



50 µg/ml



100 µg/ml

**(d) Aqueous fraction of *CC* flowers**

Another worth mentioning feature is that, despite of increasing lipid accumulation in a concentration-dependent manner, most samples showed a reduction in the size of lipid droplets at the lower concentrations 5 and 10 μg/ml.

In the same way, the exerted effects of the two *Chorisia* species on preadipocytes' differentiation exhibited a direct correlation with their polyphenol contents. This suggests that the phenolic mixtures of these species are responsible for differentiation enhancement as well as intracellular lipid accumulation, and also explains why different samples of both species displayed their effects in a concentration-dependent manner. It is also worth mentioning that total extracts of flowers and leaves of both species exhibited lower stimulatory effects on preadipocytes conversion and also DPPH scavenging abilities than their corresponding fractions especially the ethyl acetate, aqueous and chloroform parts. Such difference in potency can be attributed to the presence of the active principles in these partitions in a more concentrated form than in total extracts. Comparison of the obtained polyphenol contents of total extracts with their fractions also comes in complete harmony with this explanation.

*Chorisia* plants are known and rich sources of the flavonoid rhoifolin (Coussio, 1964) that was found to have no effect on cell growth of 3T3-L1 cells but with a stimulatory action on adiponectin secretion, phosphorylation of insulin receptor-β, and glucose transport 4 (GLUT4) translocation in differentiated adipocytes. Thus, it was suggested to exert such insulin-mimetic activity in adipocytes as a target site for this effect (Rao *et al.*, 2011). Tiliroside, another chief flavonoid biosynthesized by *Chorisia* plants (Hassan, 2009), was found to enhance fatty acid oxidation via stimulation of adiponectin signalling associated with the activation of both AMP-activated protein kinase and peroxisome proliferator-activated receptor-α (PPARα). Besides, it can ameliorate obesity-induced metabolic disorders such as hyper-insulinaemia and hyper-lipidaemia although it does not suppress body weight gain and visceral fat accumulation in obese-diabetic model mice (Goto *et al.*, 2012). On the other hand, cyanidin-3-glucoside, one of the anthocyanidins isolated from some *Chorisia* species (Scogin, 1986), was proved to promote adipocyte differentiation and glucose uptake in a dose-dependent manner. It also enhances the mRNA expression of peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), and GLUT4 in differentiated 3T3-L1 cells (Inaguma *et al.*, 2011). Members of the CCAAT-enhancer binding protein family (C/EBP-α, -β and -δ) play important roles in adipogenesis. C/EBP-β is expressed early in adipocyte differentiation program and initiates mitotic clonal expansion. In response to an adipogenic induction, C/EBP-β and -δ are first activated to promote PPARγ and C/EBP-α expression. The transcription factor PPARγ is a master regulator of adipocyte differentiation, and its activation is both necessary and sufficient for maturation of preadipocytes (Farmer, 2005; 2006). Accordingly, the richness of *Chorisia* plants in these principles may suggest their PPARγ activating properties and/or insulin-like effects, taking into account their anti-hyperglycaemic potential that was previously reported for the 70% ethanol, aqueous extracts and ethyl acetate fraction of *C. insignis* leaves (El-Alfy *et al.*, 2010). In addition, they can increase insulin-dependent glucose uptake in differentiated adipocytes and so may be beneficial in the treatment of obesity-related diseases, especially type-2 diabetes and hyperlipidaemia. Therefore, further studies will be looked-for to determine the molecular basis of such stimulatory action on adipogenesis especially for the synergistic or overall effects produced by their pool of flavonoids and other phenolics, taking into consideration the observed decrease in the size of droplets at the lower concentrations.

## 4. Conclusion

This comparative study drew an important image concerning the relationship between the polyphenol contents of *C. chodatii* and *C. speciosa* and their biological potentials including their free radical scavenging properties and effects on adipogenesis. Total ethanol extracts along with their successive fractions of various parts of both species demonstrated a concentration-dependent enhancement of 3T3-L1 preadipocyte maturation, but with a reduction of the size of the lipid droplets at the lower concentrations. In addition, these extracts showed a strong evidence of their richness in free radical scavengers. The ethyl acetate, aqueous and chloroform fractions of different

plant parts exhibited the greatest polyphenol levels, anti-radical properties and lipogenic actions, respectively. These findings collectively were found to be markedly correlated with their phenolic mixtures.

It was also clear that the polarity of the extractants, represented by different fractions, distinctly influenced the efficacy of each fraction, with the polar and flavonoids-rich ones were the most active. Conversely, the difference in plant species and their organs caused only slight differences in the produced effects. Besides, the overall results strongly suggest the potential value of these plants in obesity-related disorders and for the prevention of free radical induced diseases. On the other hand, the little phytochemical attention paid to these *Chorisia* species makes the detailed analysis of their phytochemical composition, especially of their polar extracts, along with further biological testing are strongly recommended.

## 5. Acknowledgments

The authors acknowledged that this research was supported by Japan Society of Promotion of Science (JSPS) under the Asia Africa Science Platform Program entitled: "Establishment of Integrative Research Base by Humanities and Sciences on Valorization of Useful Plants for Regional Development in North Africa". Many thanks also due to the Graduate School of Life and Environmental Sciences, Tsukuba University, Japan for providing the facilities for this study.

## 6. References

Adjanohoun, E.J. 1988. Contribution aux etudes ethnobotaniques et floristiques en Republique Populaire du Congo. ACCT, Paris, p. 605.

Ames, B.N., Gold, L.S. and Willet, W.C. 1995. The causes and prevention of cancer. *Proceedings of The National Academy of Sciences USA*, 92: 5258-5265.

Ashmawy, A.M., Azab, S.S. and Eldahshan, O.A. 2012. Effects of Chorisia crispiflora ethyl acetate extract on P21 and NF-κB in breast cancer cells. *Journal of American Sciences*, 8: 965-972.

Bailey, L.H. 1976. Hortus Third: A Concise Dictionary of Plants Cultivated in the United States and Canada. Staff of the L.H. Bailey Hortorium, Cornell University.

Blois, M.S. 1958. Antioxidant determination by the use of stable free radicals. *Nature*, 181: 1199-1200.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm-Wiss A-Technology*, 28: 25-30.

Chopra, M., Galbraith, S. and Darnton-Hill, I. 2002. A global response to a global problem: The epidemic of over nutrition. *Bulletin of World Health Organization*, 80: 952-958.

Coussio, J.D. 1964. Isolation of rhoifolin from Chorisia species (Bombacaceae). *Experientia,* 20: 562.

El-Alfy, T.S., El-Sawi, S.A., Sleem, A. and Moawad, D.M. 2010. Investigation of flavonoidal content and biological activities of Chorisia insignis H.B.K. leaves. *Australian Journal of Basic and Applied Sciences*, 4: 1334-1348.

Erding, H., Bum, K.J., Pasha, S. and Spiegelman, B.M. 1996. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. *Science*, 27: 2100-2103.

Farmer, S.R. 2005. Regulation of PPARγ activity during adipogenesis. *International Journal of Obesity*, 29: 13-16.

Farmer, S.R. 2006. Transcriptional control of adipocyte formation. *Cell Metabolism*, 4: 263-273.

Farmer, S.R. and Auwerx, I. 2004. Adipose tissue: new therapeutic targets from molecular and genetic studies: IASO stock conference 2003 report. *Obesity Research*, 5: 189-196.

Goto, T., Teraminami, A., Lee, J.Y., Ohyama, K., Funakoshi, K., Kim, Y.I., Hirai, S., Uemura, T., Yu, R., Takahashi, N. and Kawada, T. 2012. Tiliroside, a glycosidic flavonoid, ameliorates obesity-induced metabolic disorders via activation of adiponectin signaling followed by enhancement of fatty acid oxidation in liver and skeletal muscle in obese-diabetic mice. *Journal of Nutrition and Biochemistry*, 23: 768-776.

Grimble, R.F. 1994. Nutritional anti-oxidants and the modulation of inflammation: Theory and practice. *New Horizons*, 2: 175-185.

Hafez, S.S., Abdel-Ghani, A.E. and El-Shazly, A.M. 2003. Pharmacognostical and antibacterial studies of Chorisia speciosa St. Hil. flower (Bombacaceae). *Mansoura Journal of Pharmaceutical Sciences*, 19: 40-43.

Hassan, A.A. 2009. *Phytochemical and biological investigation of certain plants containing pigments*. A Thesis for the Doctor Degree submitted to Faculty of Pharmacy, Mansoura University, Egypt.

Huxley, A. 1992. *Dictionary of Gardening: The New Royal Horticultural Society*. The Macmillan Press Limited, London, the Stockton Press, New York.

Inaguma, T., Han, J. and Isoda, H. 2011. Improvement of insulin resistance by cyanidin-3-glucoside, anthocyanin from black beans through the up-regulation of GLUT4 gene expression. *BMC Proceedings*, 5: 21.

Ito, N., Hagiwara, A., Shibata, O., Ogiso, T. and Fukushima, A. 1982. Induction of squamous cell carcinoma in the fore stomach of F344 rats treated with butylated hydroxyanisole. Gann, 73, 332-334.

Johnson, P.R. and Greenwood, M.R.C. 1988. The adipose tissue. In: cell and tissue biology: A Textbook of Histology. In: Weiss, L., (Ed.), Sixth ed. Urban and Schwarzenberg, Baltimore, MD, pp. 191-209.

Kaur, G.J. and Arora, D.S. 2009. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC Complementary and Alternative Medicines, 9: 1-10.

Kirkland, J.L., Hollenberg, C.H. and Gillon, W.S. 1990. Age, anatomic site, and the replication and differentiation of adipocyte precursors. American Journal of Physiology, 258 (2 Pt 1): C206-210.

Lee, W.J., Koh, E.H., Won, J.C., Kim, M.S., Park, J.Y. and Lee, K.U. 2005. Obesity: the role of hypothalamic AMP-activated protein kinase in body weight regulation. International Journal of Biochemistry and Cell Biology, 37: 2254-2259.

Lefterova, M.I. and Lazar, M.A. 2009. New developments in adipogenesis. Trends in Endocrinology and Metabolism, 20: 107-114.

Lister, E. and Wilson, P. 2001. *Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication)*, Crop Research Institute, Lincoln, New Zealand.

Miller, N. and Rice-Evans, C. 1997. Factors influencing the antioxidant activity determined by the ABTS radical cation assay. *Free Radical Research,* 26: 195-198.

Newman, D.J. and Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, 70: 461-477.

Ngounou, F.N., Melia, A.L., Lontsia, D., Sondengama, B.L., Rahman, A.U., Choudharyb, M.I., Malik, S. and Akhtar, F. 2000. New isoflavones from Ceiba pentandra. *Phytochemistr*y, 54: 107-110.

Otto, T.C. and Lane, M.D. 2005. Adipose development: from stem cell to adipocyte. *Critical Reviews in Biochemistry and Molecular Biology*, 40: 229-242.

Pietta, P.G. 2000. Flavonoids as antioxidant. *Journal of Natural Products*, 63: 1035-1042.

Polterait, O. 1997. Antioxidants and free-radical scavengers of natural origin. *Current Organic Chemistry*, 1: 415-422.

Rao, Y.K., Lee, M-J., Chen, K., Lee, Y-C., Wu, W.S. and Tzeng, Y-M. 2011. Insulin-mimetic action of rhoifolin and cosmosiin isolated from Citrus grandis (L.) Osbeck leaves: enhanced adiponectin secretion and insulin receptor phosphorylation in 3T3-L1 cells. *Evidence-Based Complementary and Alternative Medicine*, 624375: 1-9.

Ratty, A.K., Sunamoto, J. and Das, N.P. 1988. Interaction of flavonols with 1,1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxyegenase-1. *Biochemical Pharmacol*ogy, 37: 989-995.

Ravenna, P. 1998. On the identity, validity and actual placement in Ceiba of several Chorisia species (Bombacaceae) and description of two new South American species. *Oniro*, 3: 42-51.

Refaat, J., Desoky, S.Y., Ramadan, M.A. and Kamel, M.S. 2013. Bombacaceae: A phytochemical review. *Pharmaceutical Biology*, 51: 100-130.

Roh, C. and Jung, U. 2012. Screening of crude plant extracts with anti-obesity activity. *International Journal of Molecular Sciences*, 13: 1710-1719.

Scogin, R. 1986. Reproductive phytochemistry of Bombacaceae: floral anthocyanins and nectar constituents. *Aliso*, 11: 377-385.

Slinkard, K. and Singleton, V.L. 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28: 49-55.

Tominaga, H., Kobayashi, Y., Goto, T., Kasemura, K. and Nomura, M. 2005. DPPH radical-scavenging effect of several phenylpropanoid compounds and

their glycoside derivatives. *Yakushigaku zasshi- The Pharmacological Society of Japan*, 125: 371-375.

Trease, E.G. and Evans, W.C. 1989. *Textbook of Pharmacognosy*. Third ed., Bailliere Tindal, London, pp. 81-90, 268-298.

Wagner, H. and Bladt, S. 1996. *Plants Drug Analysis: A Thin Layer Chromatography Atlas*. Second ed., Springer, Berlin, pp. 306-364.

Yun, J.W. 2010. Possible anti-obesity therapeutics from nature- A review. *Phytochemistry*, 71: 1625-1641.