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Original Article

Morphological, phytochemical and anti-hyperglycemic evaluation of *Brachychiton populneus*

Alia Y. Ragheb [a], Mona E.S. Kassem [a], Moshera M. El-Sherei [b], Mona M. Marzouk [a,*], Salwa A. Mosharrafa [a], Nabiel A.M. Saleh [a]

[a] *Department of Phytochemistry and Plant Systematics, National Research Centre, Giza, Egypt*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Giza, Egypt*

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ABSTRACT

Brachychiton populneus (Schott & Endl.) R.Br., Malvaceae, is one of five *Brachychiton* species cultivated in Egypt. Little information was found concerning the morphological, phytochemical and biological investigations of *B. populneus*. Morphological investigations of *B. populneus* were performed on fresh and dried leaves. Air-dried, ground leafy branches were extracted with 70% methanol/water yielding *B. populneus* extract. Seventeen flavonoids were isolated and identified using different chromatographic and spectroscopic techniques; eleven of them were reported for the first time from this plant. Potential activity of *B. populneus* extract against alloxan inducing oxidative stress and diabetes in male rats was preliminary investigated (four groups of ten rats /group). *B. populneus* extract (500 mg/kg bw *i.p.*) exhibited significant acute anti-hyperglycemic activity with blood glucose levels of 227.3 and 157.6 mg/dl after 4 and 24 h, respectively, compared to alloxan and standard Diamicron (5 mg/kg bw *p.o.*) groups, as well as to a normoglycemic control group at $p < 0.05$. The extract reverted the body weight values of the alloxan-induced diabetic rats to that of control animals after 24 h. In addition, *B. populneus* extract counteracted the effect of the oxidative stress induced by alloxan causing significantly increase in the glutathione content level (2.35 mmol/l) and relative decrease in the malondialdehyde level (21.31 nmol/l) and nitric oxide content (1.98 μmol/l) in serum after 24 h of treatment compared to alloxan-induced diabetic rats (1.01 mmol/l, 118.9 nmol/l, 4.69 μmol/l, respectively) and to normoglycemic control at $p < 0.05$. These effects appear to be related to the flavonoid principles. The intergeneric relationship of the genus *Brachychiton* and other related genera assessed well-supported differentiation between them. Furthermore, a significant dissimilarity was observed at interspecific level.

Introduction

Recently, in developing nations and industrialized countries the occurrence of diabetes is escalating. Imbalance between Reactive Oxygen Species (ROS) and the antioxidant defense system mediated oxidative stress is a key factor in the mechanism of several diseases including diabetes (Baynes and Thorpe, 1999). One of the possible therapies for this condition is to enhance the antioxidant resistance system by a suitable antioxidant remedy (a part from traditional treatment). Flavonoids and phenolic phytochemicals are considered to promote optimal health by protecting cellular components against free radical induced damage, due to their antioxidant and free radical scavenging effects (Dapkevicius et al., 2002). On the other hand, flavonoids have wide structural diversity, a broad variety of biological effects and have been isolated in a great scale from *Brachychiton* species (Jahan et al., 2014; El Sherei et al., 2016). They could also be used as taxonomic markers at interspecific and intergeneric levels (Crawford, 1978).

The genus *Brachychiton* Schott and Endl., Malvaceae, contains about 31 species growing wild in Australia (30 species) and New Guinea (one species) (Huxley et al., 1992). Based on some distinctive morphological features, that genus with five other genera (*Firmiana* Marsili, *Hildegardia* Schott and Endl., *Pterocymbium* R.Br., *Pterygota* Schott and Endl. and *Scaphium* Schott and Endl.) was treated under the genus *Sterculia* (El Sherei et al., 2016). Some biological activities have been reported for a number of species belonging to the genus *Brachychiton, viz*; antidiabetic (Desoky and Youssef, 1997; Kassem et al., 2002; Abou Zeid et al., 2017), digestive

* Corresponding author.
E-mail: mm.marzouk@nrc.sci.eg (M.M. Marzouk).

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system and urinary tract disorders (Keay, 1989), antischistosomal (Yousif et al., 2007), antimicrobial (Newbold et al., 1997; Yousif et al., 2007; Abdel-Megeed et al., 2013), antioxidant (Abdel-Megeed et al., 2013) and anti-inflammatory (Agyare et al., 2012).

Five *Brachychiton* species are cultivated in Egypt; *B. acerifolius* (Cunn. ex G. Don) Macarthur, *B. australis* (Schott and Endl.) A. Terrac., *B. discolor* F. Muell., *B. populneus* (Schott and Endl.) R. Br. and *B. rupestris* (T. Mitch. ex Lindl.) K. Schum. *B. populneus* [Synonyms: *Sterculia diversifolia* G. Don., *B. populneus subsp. populneus* (Schott and Endl.) R. Br. and *Poecilodermis populnea* Schott and Endl] is a small to medium sized ornamental tree, cultivated in gardens and roadsides and commonly known as "Kurrajong or Bottelboom Tree". The bark was used as fiber, and the soft spongy wood for making shields. The leaves are also used as emergency fodder for drought-affected animal stock. The seeds are used as a coffee supplement by roasting and crushing (Anderson, 2016). Petronici et al. (1970) identified eight fatty acids (palmitic, heptadecenoic, stearic, oleic, linoleic, eicosenoic, malvalic and sterculic acids) in *B. populneus* fruit, while Desoky and Youssef (1997) reported the isolation of three flavonol aglycones (kaempferol, quercetin and isorhamnetin) and five flavonol glycosides (kaempferol 3-*O*-β-glucoside, kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-(2″-rhamnosylrutinoside), isorhamnetin 3-*O*-rutinoside and quercetin 3-*O*-arabinoside) from the stem bark. Recently, Batool et al. (2018) specified rutin, catechin and myricetin and proposed the presence of antioxidant and hepatoprotective mediators in the methanol extract of leaves. Except for these publications, no previous phytochemical or biological investigations have been reported for *B. populneus*. Additionally, there are no micro-morphological and chemosystematic surveys concerning the studied species.

Accordingly, it was deemed of interest to evaluate the morphological features and detailed flavonoid investigations which could play a role in supporting the classification status, as well as, anti-hyperglycemic evaluation.

Material and methods

General

^{1}H NMR experiments were recorded on a Jeol EX-500 spectrometer: 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR). UV:UV spectrophotometer (Shimadzu UV-240). EI–MS: Finnigan-Mat SSQ 7000 spectrometer. Oxidative stress biomarkers were determined using double beam spectrophotometer (Schimadzu, Japan). Column chromatography; Polyamide S6 (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) using MeOH/H_2O as eluent. Paper chromatography (descending); Whatman No. 1 and 3 MM papers. Sephadex LH-20 (Pharmacia). Authentic samples were obtained from the Phytochemistry and Plant Systematics Department, NRC. EOS Canon camera. Olympus Microscope, CX 41. Olympus digital camera, E-330. OK glucometer (Lifescan, Milpitas, CA).

Plant material

Fresh leafy branches of *Brachychiton populneus* (Schott & Endl.) R.Br., Malvaceae, were collected in March, 2012 from Orman Botanical Garden, Giza, Egypt. The plant was kindly identified by Dr. M. El Gibali, former researcher of botany, National Research Centre (NRC), Egypt. A voucher specimen was kept at the Herbarium of the Department of Pharmacognosy No. (21-12-2016 II), Faculty of Pharmacy, Cairo University.

Botanical study

The macromorphological investigations were described from fresh materials or after keeping in 70% ethanol containing 5% glycerin by naked eye, with a hand lens or low magnification using a stereomicroscope. The leaves were air dried, reduced to fine powder and kept for microscopic examination. For detailed micromorphological studies, mature leaves were fixed in FAA (40% formaldehyde solution, acetic acid, 70% ethanol (5:5:90). Transverse sections of lamina and petiole were investigated through hand-microtome cross sections at 8–10μ. The sections were stained with safranin-light green or crystal violet-erythrosine combination according to the conventional methods (Abd-Elgawad and Alotaibi, 2017), ruthenium red for testing mucilage and $FeCl_3$ for testing tannins (Pakravan et al., 2007). Epidermal peels of upper and lower surfaces were made. They were stained in 1% aqueous safranin solution 4–8 min, washed in water to remove excess stain before mounting on clean slides in 10% glycerol (Metcalfe and Chalk, 1979).

Extraction, isolation and structure elucidation

Air-dried, grounded leafy branches of *B. populneus* (1.7 kg) were extracted three times by repeated percolation (40–60 °C) with 70% methanol/water till exhaustion then evaporated under reduced pressure affording 80 g *B. populneus* extract (BPE). BPE (70 g) was defatted with petroleum ether (40–60 °C) then subjected to a polyamide column (150 × 5 cm). Stepwise gradient elution was carried out starting with water as eluent then decreasing the polarity by increasing the concentration of methanol (Mabry et al., 1970). Fractions of 100 ml each were collected. Similar fractions were combined according to their paper chromatography (PC) properties using H_2O, 15% HOAc (H_2O–HOAc 85:15) and BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper layer) as eluents to give six main fractions (A-F). Fraction A (100% H_2O; 11 g) was re-chromatographed over a Sephadex chromatographic column (CC) (35 × 2.5 cm). Elution was carried out using a solvent system of methanol/water (1:1 v/v). It was subjected to preparative paper chromatography (PPC) twice using 15% HOAc as eluent, then on a Sephadex LH-20 column (35 × 2.5 cm) using MeOH to yield compounds **1** (42 mg) and **2** (49 mg). Fraction B (20% MeOH/H_2O; 8.5 g) was re-chromatographed over a Sephadex CC (35 × 2.5 cm) using H_2O followed by PPC using BAW as eluents, then on a Sephadex LH-20 column (35 × 2.5 cm) using MeOH to yield compound **3** (23 mg). Fraction C (40% MeOH/H_2O; 10.5 g) was subjected to Sephadex CC (35 × 2.5 cm) using H_2O as eluent. It was purified by PPC using BAW (double solvent) followed by 15% HOAc, then on a Sephadex LH-20 column (35 × 2.5 cm) using MeOH to yield compounds **4** (39 mg), **5** (52 mg), and **6** (47 mg). Fraction D (60% MeOH/H_2O; 14 g) was subjected to PPC using BAW as eluent. Subsequently, it was applied to Sephadex CC (35 × 2.5 cm) using methanol/water (1:1 v/v) as eluent. The isolated compounds were further purified by re-chromatography on Sephadex LH-20 column (60 × 1.5 cm) using methanol as eluent yielding compounds **7** (8 mg) and **8** (11 mg). Fraction E (80% MeOH/H_2O; 11.5 g) yielded compounds **9** (48 mg), **10** (18 mg), **11** (11.5 mg) and **12** (19 mg) by applying it to a Sephadex CC (35 × 2.5 cm) using methanol/water (1:1 v/v) as eluent. Fraction F (100% MeOH; 13 g) was also chromatographed on PPC using BAW followed by 50% HOAc then on Sephadex LH-20 column (35 × 2.5 cm) using MeOH to yield compounds **13** (7.5 mg), **14** (18 mg), **15** (11 mg), **16** (16 mg) and **17** (6 mg).

The structures of the compounds were elucidated using R*f* values, colour reactions, chemical methods (acid hydrolysis and $FeCl_3$ oxidative hydrolysis) as well as spectral analysis (UV, MS and NMR). Further confirmation was performed through comparing with authentic samples and/or published data. Acid hydrolysis for

Fig. 1. Photographs of *Brachychiton populneus* (Schott & Endle.) R. Br. (A) Whole tree in its natural habitat (X = 0.0058), a close up view to flowers (X = 0.2), (B) alternate arrangement of the leaves (X = 0.135), (C) the upper surface of the leaf (X = 0.39), (D) the lower surface of the leaf (X = 0.39), (E) flowers; 1, male flower, 2, female flower (X = 0.14), F) fruits in star-like cluster (X = 0.25), (G) opened fruit (X = 0.47), (H) seeds (X = 0.75).

O-glycosides (2 N HCl, 2 h, 100 °C) were carried out and followed by co-PC with authentic samples to identify the aglycones and sugar moieties. The sugar units of *C*-glycosyl flavonoids were determined using ferric chloride oxidative hydrolysis (20% $FeCl_3$, 6 h) followed by co-PC with a standard sugar mixture using BBPW (benzene, *n*-butane, pyridine, water (1:5:3:3)) as eluent (Mabry et al., 1970).

Biological assay

Animals

Ten albino mice (weighing 25–30 g) were used for determination of LD_{50} and forty male albino rats, eight weeks old (weighing 120–150 g) obtained from the breeding colonies of the National Research Centre (Dokki, Giza, Egypt), and acclimatised with free access to food (standard laboratory pellets of 20% protein, 5% fats, and 1% vitamins) and tap water for at least one week at room temperature at 23–25 °C. The animals were fasted for 24 h before induction of hyperglycemia but allowed free access to water. All animal procedures were performed after approval by the National Research Centre Medical Ethics Committee (09085) and in accordance with the recommendations of the proper care and use of laboratory animals.

Experimental design

Ten healthy rats were treated with saline and served as a normoglycemic group (Control). Diabetes was induced by a single

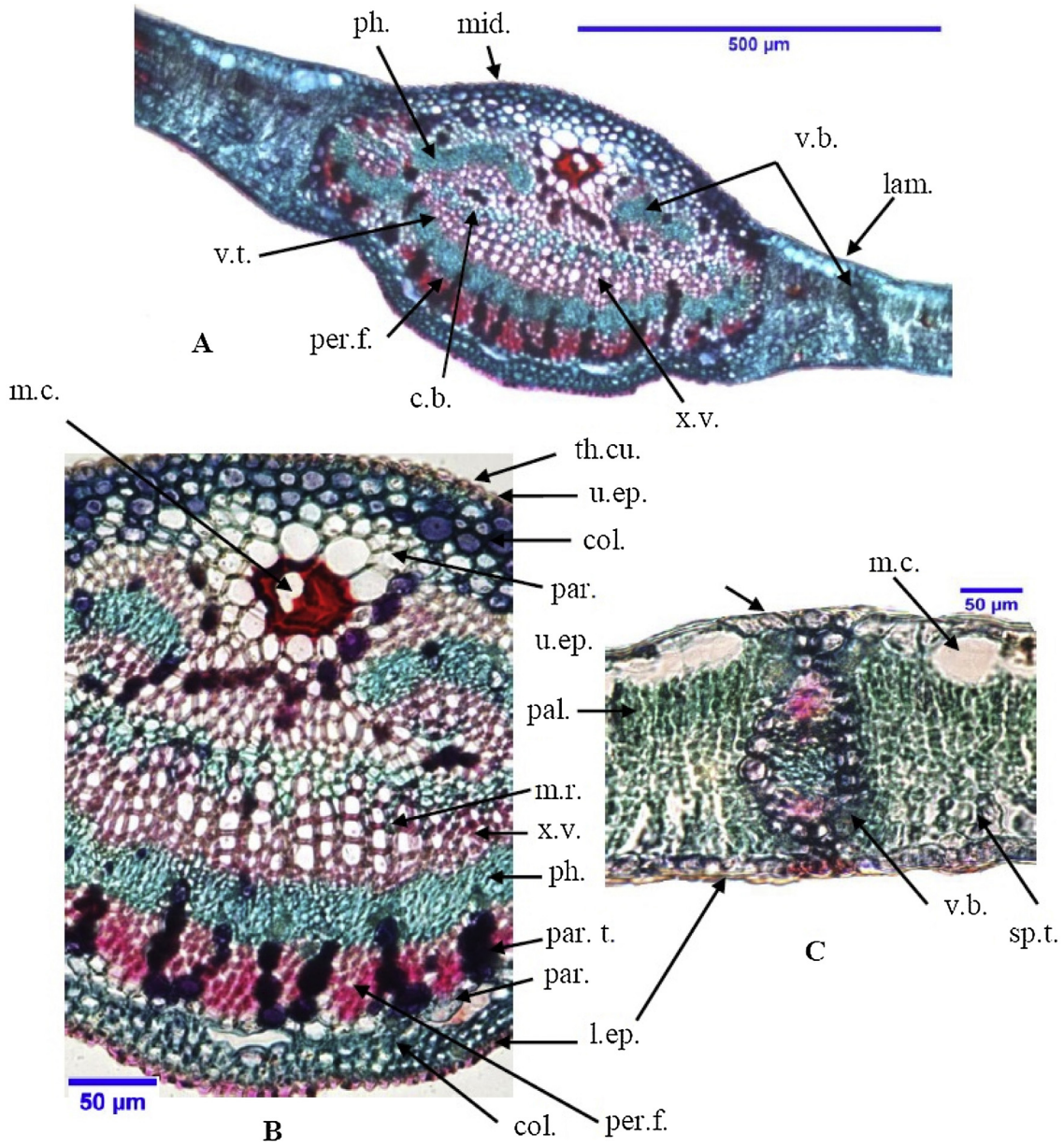


Fig. 2. Micromorphology of the lamina of *Brachychiton populneus* (Schott & Endle.) R. Br. (A) T.S. of the lamina (X = 100), B) detailed T.S. in the leaf showing midrib region (X = 240), C) detailed T.S. in the leaf showing lamina region (X = 160), c.b.; central bundles, col.; colenchyma, l.ep.; lower epidermis, lam.; lamina, m.c.; mucilage cavity, m.r.; medullary rays, mid.; midrib, pal.; palisade cells, par.; parenchyma, par. t.; parenchyma with tannin content, per.f.; pericyclic fibers, ph.; phloem, sp.t.; spongy tissue, th.cu.; thick cuticle, u.ep.; upper epidermis, v.b.; vascular bundles, v.t.; vascular tissue, x.v.; xylem vessel.

injection of a freshly prepared alloxan monohydrate (Sigma, No. 242-646-8) according to rat weight at dose (150 mg/kg, *i.p.*) to overnight-fasted rats (Rao et al., 2001). After a period of three days the rats which did not develop more than 200 mg/dl glucose levels, were rejected. The alloxan-induced diabetic rats were further classified into three groups with ten rats in each group. Group 1 received saline and served as hyperglycemic group. Group 2 received Diamicron (5 mg/kg, *p.o.*) and was given as standard group. Group 3 received BPE (500 mg/kg, *i.p.*). After injection of BPE, blood samples were withdrawn from the tail vein of the rats at zero, 4 and 24 h. The collected samples were kept into covered test tubes.

Determination of blood glucose level (BGL)

The blood glucose level was determined by the glucose-oxidase principle (Beach and Turner, 1958) using the OK glucometer and results were reported as mg/dl (Rheney and Kirk, 2000).

Oxidative stress biomarkers

Serum was separated from blood samples (at 24 h) by centrifugation at 1008*g*-force for 10 min at 4 °C. The clear sera were immediately stored at −70 °C in a clean plastic eppendorf for the subsequent determination of glutathione content (GSH), malondialdehyde level (MDA) and nitric oxide content (NO).

Determination of serum reduced glutathione content. Reduced glutathione content of serum was measured according to method of Bulaj et al. (2002). In centrifuge tubes containing 0.5 ml of precipitating solution (10% trichloroacetic acid-6 mM Na_2EDTA), 50 µl of serum was added, vortexed and centrifuged at 448*g*-force for 5 min. 0.1 ml of the resulting clear supernatant was added to 1.85 ml of potassium phosphate buffer (100 mM, pH 8) and 0.1 ml of Ellman's reagent [5,5′-dithiobis (2-nitrobenzoic acid)] and mixed thoroughly. After 5 min, the absorbance was measured

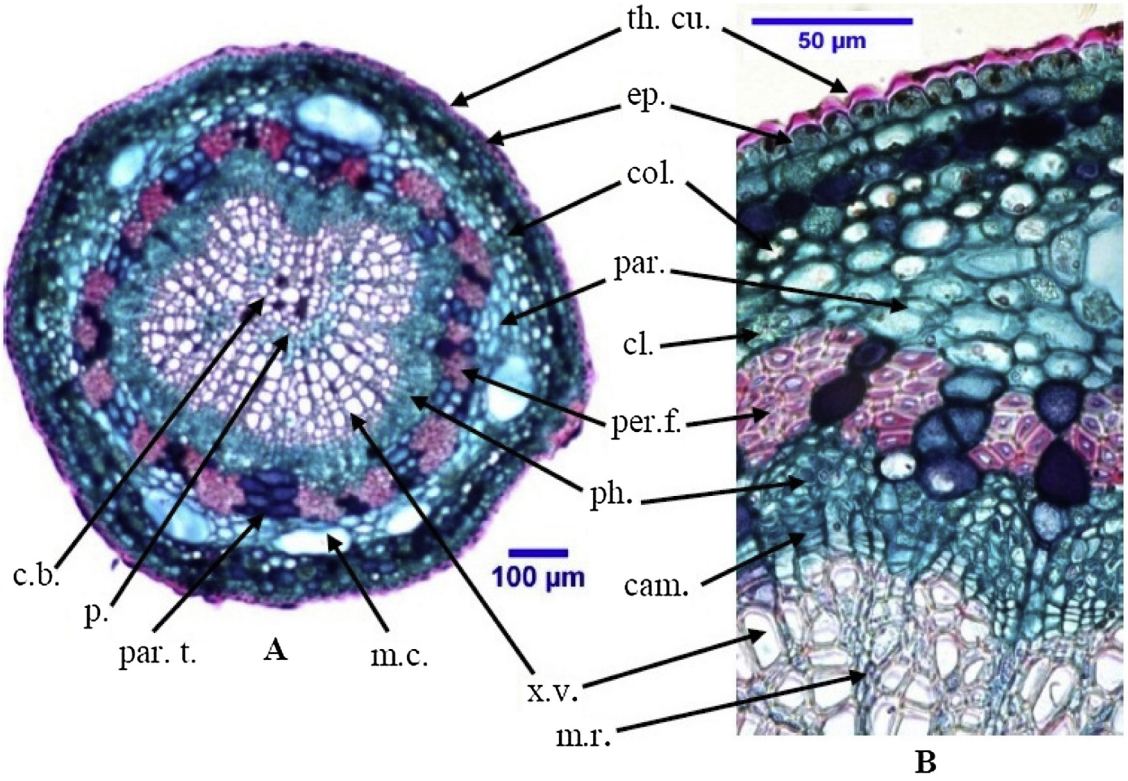


Fig. 3. Micromorphology of the petiole of *Brachychiton populneus* (Schott & Endle.) R. Br. (A) T.S. of the petiole (X = 70), (B) detailed T.S. in the petiole (X = 400), cam; cambium, c.b.; central bundles, cl.; clusters of calcium oxalate, col.; collenchyma, ep.; epidermis, m.r.; medullary rays, m.c.; mucilage cavity, p.; pith, par.; parenchyma, par. t.; parenchyma with tannin content, per.f.; pericyclic fibers, ph.; phloem, th.cu.; thick cuticle, x.v.; xylem vessel.

at 412 nm using a double beam spectrophotometer (Schimadzu, Japan) against reagent blank. The GSH level in serum was expressed as mmole/l and calculated from the following formula: GSH content (mmole/l) = At/As × Cs × dilution factor $\frac{At}{As} \times Cs \times$ dilutionfactor, Where: At = absorbance of test sample, As = absorbance of standard and Cs = concentration of standard.

Determination of thiobarbituric acid reactive substances

Lipid peroxide levels were measured in serum as thiobarbituric acid reactive substances (TBARS). Malondialdehyde (MDA), one of the degradation products of lipid peroxides, was used as standard (Uchiyama and Mihara, 1978). In centrifuge tubes containing 0.5 ml of precipitating solution (10% trichloroacetic acid-6 mM Na_2EDTA), 50 µl of serum was added, vortexed and centrifuged at 448*g*-force for 5 min. 0.5 ml of the resulting clear supernatant was added to 3 ml of *ortho*-phosphoric acid (1%, v/v) and 1 ml of thiobarbituric acid (0.67%, w/v) then heating for 20 min at 100 °C, the mixture was cooled and 4 ml of *n*-butanol were added and mixed vigorously, then separated by centrifugation at 1008 × *g*-force for 10 min. Optical density was measured against reagent blank at 532 nm using a double beam spectrophotometer (Schimadzu, Japan). TBARS concentration in serum was expressed as nmole MDA/l and calculated from the following equation: TBARS (nmole/l) = ODt/ODs × Cs × dilution factor, Where: OD: Optical density, Cs: Concentration of standard malondialdehyde (MDA) solution.

Determination of nitric oxide content. Nitric oxide (NO), an unstable reactive nitrogen free radical, was determined in the present study using biochemical method of Montgomery and Dymock (1961) where the production of NO is expressed by endogenous nitrate/nitrite metabolites. In a test tube, 0.25 ml of zinc sulphate (10%) was added to 0.25 ml serum and left for 15 min to deproteinize it. Samples were then centrifuged at 16,128*g*-force for 20 min using cooling centrifuge (Hermle, Germany). 250 µl of NEDD reagent [*N*-(1-naphthyl) ethylenediaminedihyrochloride 0.1% (w/v) in distilled water] was added to 250 µl of the obtained supernatant, and incubated at 37 °C for 10–15 min afterward 250 µl of sulphanilamide solution [2% (v/v) in distilled water] was added and incubated at 37 °C for 10–15 min. The mixture was cooled and the absorbance of the pink coloured chromophore was measured at 540 nm using a double beam spectrophotometer (Schimadzu, Japan) against a reagent blank where 250 µl distilled water was used instead of the sample. The level of total nitrite/nitrate (NO_x) was expressed as µmol/l and was calculated by: NOx (µmol/l) = At/As × Cs × dilution factor, where At: absorbance of the test sample, As: absorbance of the standard sample and Cs: concentration of standard.

Statistical analysis

All values are presented as means ± SEM (standard error of the means) for ten rats in each group. Comparison between groups was carried out using the non-parametric one-way analysis of variance (ANOVA) followed by Tukey-HSD multiple comparisons test to judge the difference between various groups. Difference was considered significant when $p < 0.05$. Graph pad software (version 7.00) and Excel 10 were used to carry out these statistical tests and plot graphs.

Results and discussion

Botanical study

Macro-morphological study

Brachychiton populneus is a bottle-shaped taper like tree, with dark brown bark and crowded leaves at the end of branches mainly

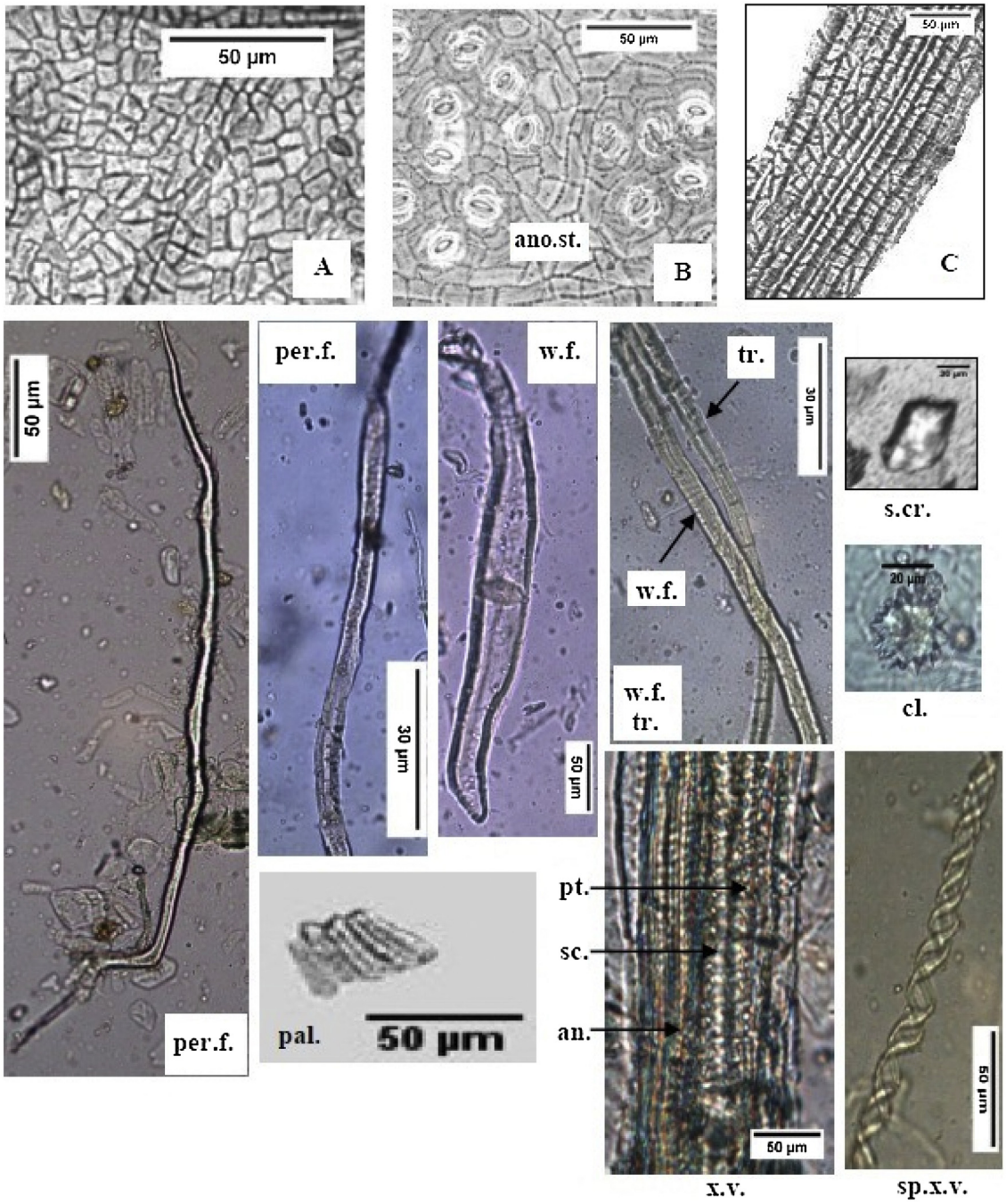


Fig. 4. Powdered leaf of *Brachychiton populneus* (Schott & Endle.) R. Br. (A) Fragment of upper epidermis (X = 400), (B) fragment of lower epidermis showing anomocytic stomata (X = 280), C) fragment of neural epidermal cells (X = 160), an; annular xylem vessel, ano.st.; anomocytic stomata, cl.; clusters of calcium oxalate (X = 350), pal., palisade cells (X = 400), per.f.; pericyclic fiber (X = 260,700), pt.; pitted xylem vessel, s.cr.; solitary crystal (X = 133), sc.; scalariform xylem vessel, sp.x.v.; spiral xylem vessel (X = 300), tr.; tracheid, w.f.; wood fiber (X = 180, 533), x.v.; xylem vessel (X = 180).

Table 1
Effect of *Brachychiton populneus* extract on blood glucose level (BGL) and body weight of alloxan-induced diabetic rats.

Treatments	BGL (mgdl)			BW (g)		
	0 h	4 h	24 h	0 h	24 h	% changes
Normoglycemic	97.8 ± 4.49	94.60 ± 8.16	95.00 ± 6.78	128.33 ± 3.15	147 ± 2.23	14.39 ± 2.09
Hyperglycemic	551.10 ± 22.90[b]	488.33 ± 46.12[b]	448.50 ± 43.07[b]	127.5 ± 0.92	108.33 ± 5.57[a,c]	15.07 ± 4.15
Standard control (Diamicron)	502 ± 20.08[b]	405.42 ± 24.08[b]	161.42 ± 11.15[a]	126.33 ± 3.62	138.925 ± 1.7[b]	9.97 ± 3.70
BPE	387.5 ± 42.11[b,a]	227.3 ± 30.28	157.6 ± 16.57[b,a]	121 ± 3.26	122.73 ± 4.67[a,b]	1.43 ± 2.25

Values are expressed as mean ± SEM, n = 10.Values are statistically significant at $p < 0.05$.

[a] Significant from saline normoglycemic control.

[b] Significant from Alloxan hyperglycemic control

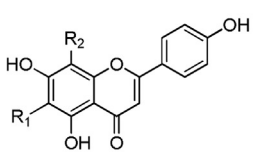
[c] Significant from diamicron standard control.

distributed in coastal and sub-coastal areas (Fig. 1A). Leaves are simple, grayish-green in colour, glabrous and glossy, cauline, alternate, exstipulate, petiolate (2–5 cm). It is ovate to broadly ovate with acuminate apex (5–11 cm in length and 2–6 cm in width), sinuate margine and cuneate base, showing pinnate reticulate venations and having papery to coriaceous texture, no odour and a characteristic taste (Fig. 1B–D). Inflorescence is terminal or short axillary panicles, 12–15 cm long, 10–15 flowered (Fig. 1A). Flowers are pedicellate (0.3–1.5 cm), epicalyx absent, calyx consists of five lanceolate sepals with entire margin and acuminate apex, off-white with reddish dots inside in colour, slightly pubescent outside, glabrous inside. The sepals united and form bell shape (campanulate calyx), corolla absent. Androecium is formed of ten yellow, glabrous anthers, showed as a capitate-globose cluster on a white, glabrous androphore (male flower). Gynoecium is consisting of ovoid, sessile, tomentose ovary, with a ring of ten vestigial anthers at its base, 5-carpels, axial placentation with long and tomentose style and curved and lobed stigma (female flower) (Fig. 1E). Fruits are dark brown woody follicles (5–12) in star-like clusters, pubescent, dehiscent, boat-shaped, with a size of (4–7) × (1–2.5) cm and (3–6) cm woody stalk (Fig. 1F, G). Seeds are ovoid and angular in shape with hairy surface that may cause skin and eye irritation, brownish yellow in colour, ranged from 10 to 15 with dimensions of (0.5–0.8) × (0.2–0.5) cm (Fig. 1H).

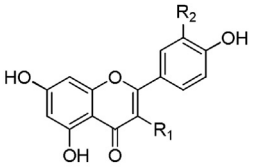
Micromorphological study

Lamina. The transverse section of lamina appeared biconvex slightly more prominent in the lower (Fig. 2A). The upper and lower epidermises are devoid from the glandular and non-glandular trichomes. The leaf displayed a dorsiventral mesophyll interrupted in the midrib region with collenchymatous cells. Idioplasts containing calcium oxalate clusters and prisms, mucilage (cavities) and tannin (cells) are present. Upper epidermis is formed of one row of radially elongated cells with thick cuticle, as seen in the transverse section (Fig. 2A, C). In surface view, the cells appeared polygonal, nearly isodiametric with straight to slightly wavy anticlinal walls, covered with faintly striated cuticle and showing no stomata (Fig. 4A). Lower epidermis is formed of one row of radially elongated cells with thick cuticle, as observed in the transverse section (Fig. 2A, C). In surface view, the cells appeared polygonal, nearly isodiametric with straight to slightly wavy and beaded anticlinal walls, covered with faintly striated cuticle and showing anomocytic stomata. The stomata are with wide osteoles and surrounded by 3–5 subsidiary cells (Fig. 4B). Neural epidermis (upper and lower) composed of radially elongated cells; the lower neural epidermal cells are smaller in size compared to the upper ones covered with thick cuticle, as viewed in the transverse section (Fig. 2A, B). In surface view, it showed polygonal, axially elongated cells with straight and beaded anticlinal walls. They are covered with faintly striated cuticle and devoid of stomata (Fig. 4C). Mucilage was detected in most of the epidermal cells (ruthenium red test). Minute clusters and prisms of calcium oxalate crystals were rarely seen. Mesophyll is heterogeneous and dorsiventral composed of 2–3 layers of palisade cells discontinuous in the midrib region, occupying half of the leaf thickness. The cells are closely packed, radially elongated columnar, containing choloroplasts, with thin and straight anticlinal walls. Lower layers are shorter than the upper ones abutting the upper epidermis. The spongy tissue is formed of 3–5 layers of rounded, thin walled and slightly irregular chlorenchyma cells with intercellular spaces, occupying half of leaf thickness. Few calcium oxalate clusters and prisms are dispersed through. Small collateral vascular bundles are scattered in the mesophyll in the small lateral veins (Fig. 2C). Cortical tissue of the midrib region (Fig. 2B) in the upper region shows 2–3 rows of subepidermal collenchyma cells followed by 8–12 rows of parenchyma cells. The lower layer consists of 1–2 rows of subepidermal collenchyma cells followed by 2–3 rows of parenchyma cells. The collenchymatous cells are small rounded cells with thick cellulosic walls and no intercellular spaces while the parenchymatous cells appeared large, rounded with thin cellulosic walls and narrow intercellular spaces. The endodermis is parenchymatous and indistinct. Pericycle is sclerenchymatous; formed of patches of lignified and non-lignified fibers encircling the vascular tissue. The fibers are long showing wide lumina and blunt apices (Fig. 2B, 4). Vascular stele (Fig. 2A, B) appeared as crescent shaped arch extended to the lower side and another two smaller upper inverted ones of collateral type showing the phloem towards outside and the xylem inside. 2–3 Small central collateral bundles surrounded by strands of non-lignified sclerenchymatous fibers are present. Phloem consists of sieve elements, companion cells and phloem parenchyma. Cambium is undifferentiated. The xylem is formed of vessels, wood parenchyma and wood fibers. The vessels have annular, spiral, scalariform and pitted thickenings (Fig. 4). The wood parenchyma is rectangular, slightly elongated cells, with slightly thin pitted walls (Fig. 4). The wood fibers are fusiform, having slightly thick, lignified walls, wide lumina and blunt apices (Fig. 4). The medullary rays are uni-, bi- and multi-seriate of rectangular thin walled cellulosic parenchyma cells (Fig. 4). Calcium oxalate clusters and prisms are few, scattered in the outer and inner ground tissues and phloem. Parenchymatous cells containing tannins ($FeCl_3$ test) are present. Mucilage cavities (idioblast) are present in the upper outer ground tissue and lamina (Fig. 2A, B).

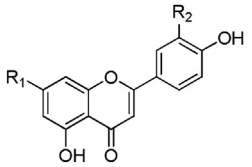
Petiole. The petiole transverse section is nearly circular in outline (Fig. 3A). Epidermis followed by a relatively narrow cortex surrounding a closed ring of collateral vascular bundles capped by patches of pericycle fibers. The pith is relatively narrow with 5–6 fine central vascular bundles. Epidermis resemble that of neural region, composed of radially elongated cells with straight anticlinal walls, covered with faintly striated cuticle and showing no stomata. Cortical tissue consists of 2–4 rows of small rounded collenchymatous cells with thick cellulosic walls and no intercellular spaces followed by 3–7 large polygonal to oval parenchymatous cells with thin cellulosic walls and narrow intercellular spaces. Mucilage cavities (5–7) are scattered. Calcium oxalate clusters and prisms are Frequent. Parenchymatous cells containing tannins are present (Fig. 3). The Endodermis is parenchymatous and indistinct. Pericycle appeared as incomplete ring interrupted with parenchyma cells, sclerenchymatous; formed of strands of long lignified fibers encircling the vascular tissue with wide lumina and blunt apices (Fig. 4). Vascular stele appeared as an interrupted closed ring of collateral vascular bundles, showing the phloem directed to the outer side and the xylem extended towards inner one. 5–6 Small collateral central bundles were scattered (Fig. 3A). The phloem is formed of sieve elements, companion cells and phloem parenchyma. The cambium is represented by 3–4 rows of tangentially elongated, thin walled meristematic cells (Fig. 3B). The xylem is formed of vessels, tracheids, wood parenchyma and wood fibers. The vessels are annular, spiral, pitted and scalar form. The wood parenchyma is polygonal elongated cells, having wide lumina with slightly pitted lignified walls. The wood fibers are fusiform having slightly thick, lignified walls, wide lumina and blunt to pointed apices (Fig. 4). The medullary rays are uni-, bi- and multi-seriate of rectangular thin walled cellulosic parenchyma cells. Pith is formed of 5–7 rows of large thin walled rounded parenchyma cells and frequent calcium oxalate clusters and prisms. Parenchymatous cells containing tannins are present. 8–10 scattered mucilage cavities are also present (Fig. 3A).



1 R_1=H; R_2=*C*-glucopyranoside
2 R_1=*C*-glucopyranoside; R_2=H
3 R_1=R_2=*C*-glucopyranoside



4 R_1=*O*-rutinopyranoside; R_2=OCH_3
5 R_1=*O*-rutinopyranoside; R_2=OH
6 R_1=*O*-rutinopyranoside; R_2=H
9 R_1=*O*-rhamnopyranoside; R_2=OH
10 R_1=*O*-glucopyranoside; R_2=H
13 R_1=OH; R_2=OCH_3
14 R_1=R_2=OH
17 R_1=OH; R_2=H



7 R_1=*O*-glucuronide; R_2=OH
8 R_1=*O*-glucuronide; R_2=H
11 R_1=*O*-glucopyranoside; R_2=OH
12 R_1=*O*-glucopyranoside; R_2=H
15 R_1=OH; R_2=H
16 R_1=R_2=OH

Phytochemical investigation

Isolation and structural elucidation of flavonoid compounds

Seventeen flavonoid compounds were isolated and identified from the leafy branches of *B. populneus*. They were identified as apigenin 8-*C*-β-glucopyranoside (vitexin **1**) (Marzouk et al., 2008), apigenin 6-*C*-β-glucopyranoside (isovitexin **2**) (Peng et al., 2008), apigenin 6,8-di-*C*-β-glucopyranoside (vicenin **3**) (Hussein et al., 2009), isorhamnetin 3-*O*-β-rutinoside (**4**) (Bragg et al., 1978), quercetin 3-*O*-β-rutinoside (rutin **5**) (Marzouk et al., 2016), kaempferol 3-*O*-β-rutinoside (**6**) (Marzouk et al., 2016), luteolin 7-*O*-β-glucuronide (**7**) (El Sherei et al., 2018), apigenin 7-*O*-β-glucuronide (**8**) (El Sherei et al., 2018), quercetin 3-*O*-α-rhamnopyranoside (quercetrin **9**) (Kassem et al., 2013), kaempferol 3-*O*-β-glucopyranoside (astralagin **10**) (Marzouk et al., 2012), luteolin 7-*O*-β-glucopyranoside (**11**) (Marzouk et al., 2008), apigenin 7-*O*-β-glucopyranoside (**12**) (El Sherei et al., 2018), isorhamnetin (**13**) (Hussein et al., 2009), quercetin (**14**) (Marzouk et al., 2010), apigenin (**15**) (El Sherei et al., 2018), luteolin (**16**) (El Sherei et al., 2018), and kaempferol (**17**) (Kawashty et al., 2012). Ten compounds (**1**-**3**, **7**-**9**, **11**, **12**, **15** and **16**) were isolated for the first time from the plant species *B. populneus*.

Chemosystematic significance

Chemotaxonomic studies constitute one of the most important methods of determining the taxonomic positions of taxa. From the interspecific point of view, five of approximately 31 accepted species of *Brachychiton*, including the present one, are known to produce around 27 flavonoid compounds belonging to four classes containing seven flavones, fifteen flavonols, three *C*-glycosyl flavones and two anthocyanins (Table 3). The flavones are characteristic of *B. populneus* and *B. acerifolius*; they are represented as apigenin and luteolin along with their 7-*O*-glucoside and 7-*O*-glucuronide (De Laurentis et al., 2003 and Farag et al., 2015). Only one flavone diglycosides (apigenin 7-*O*-(2″-α-rhamnoside)-*O*-β-glucuronide) was recently reported for *B. acerifolius* (Abou Zeid et al., 2017).

The flavonols are recognised in the five species and are represented as four aglycones (kaempferol, quercetin, rhamnetin and isorhamnetin), and eleven glycoside derivatives of kaempferol, quercetin and isorhamnetin. Quercetin aglycone is common among the five *Brachychiton* species (Desoky and Youssef, 1997; Kassem et al., 2002; De Laurentis et al., 2003; Kassem, 2007; Farag et al., 2015), while kaempferol and isorhamnetin were reported for *B. populneus*, *B. acerifolius* and *B. rupestris* (Desoky and Youssef, 1997; De Laurentis et al., 2003; Farag et al., 2015) as well as rhamnetin which is characteristic for *B. discolor* (Kassem, 2007). The glycosylation patterns of flavonols were generally represented at position 3. They are reported as 3-*O*-monoglycoside of kaempferol and quercetin, 3-*O*-diglycosides (rutinoside) of kaempferol, quercetin and isorhamnetin as well as 3-*O*-triglycosides of kaempferol and isorhamnetin (Table 3). Kaempferol 3-*O*-glucoside and quercetin 3-*O*-arabinoside are characteristic for *B. populneus* (Desoky and Youssef, 1997), while quercetin 3-*O*-glucoside is characteristic for *B. australis* only (Kassem et al., 2002) and quercetin 3-*O*-galactoside for *B. acerifolius* (De Laurentis et al., 2003). Moreover, quercetin 3-*O*-rhamnoside was reported in *B. discolor* (Kassem, 2007). The 3-*O*-rutinoside as well as 3-*O*-(2″-rhamnosyl-rutinoside) of kaempferol and isorhamnetin were isolated from *B. populneus* and *B. rupestris*, while the 3-*O*-(2″-rhamnosyl robinoside) of kaempferol and isorhamnetin were characteristic for *B. rupestris* (Desoky and Youssef, 1997). Furthermore, quercetin 3-*O*-rutinoside was detected in *B. populneus*, *B. acerifolius* and *B. australis* while isorhamnetin 3-*O*-rutinoside was characteristic for *B. populneus*, *B. rupestris* and *B. australis* (Kassem et al., 2002; De Laurentis et al., 2003; Farag et al., 2015).

C-glycosyl flavones were common in *B. populneus* and represented as vitexin, isovitexin and vicenin, the later compound was also found in *B. acerifolius* along with two anthocyanin glycosides (Farag et al., 2015).

Hence, the flavonoid profiles of the five *Brachychiton* species growing in Egypt may provide useful taxonomic differences at the infraspecific level, where *B. populneus* and *B. acerifolius* sharing three flavonoid groups (flavones, flavonols and *C*-glycosyl flavones) which differ from the other species by sharing a flavonol group only. *B. acerifolius* is also characterized by the synthesis of anthocyanin nuclei.

At the intergeneric level, no report regarding the flavonoid constituents of *Pterocymbium* and the survey of the other genera (*Brachychiton, Firmiana*, *Hildegardia*, *Pterygota*, *Scaphium* and *Sterculia*) showed a wide range of flavonoid compounds (Table S1). They occurred commonly as glycosides of flavones and flavonols. The flavone glycosides are present as 7-*O*-glucoside and/or 7-*O*-glucuronide of apigenin and luteolin distributed among the species of *Brachychiton*, *Pterygota* and *Sterculia,* while those of chrysoeriol are characteristic for genus *Sterculia* (Xia, 2009; Hossain et al., 2012). The 6- or 8-hydroxyflavones (scutellarein, isoscutellarein, 6-hydroxyluteolin and hypolaetin) were also detected and characteristic for *S. foetida*.

Glycosylation of flavonols were often substituted on the OH group at position 3 for kaempferol, quercetin and/or isorhamnetin. Members of the genus *Firmiana* are characterized by mono and/or di-glycosides of kaempferol and/or quercetin, but the 7-*O*-glucoside of kaempferol and isorhamnetin were reported for *Scaphium scaphigerum* (G. Don) Guib. and Planch. (Petchlert et al., 2012). The only flavonol-*O*-acyl glycoside derivative; kaempferol 3-*O*-β-(4″-*p*-coumaryl)-glucopyranoside was recorded in *P. alata* (El Sherei et al., 2018). On the other hand, two tetra methylated derivatives of quercetin were reported in *S. foetida* only (Anjaneyulu and Murty, 1981).

C-glycosylflavones occurred as mono- and di-glycosides. Two mono-*C*-glucosides of apigenin were recorded: vitexin in *Sterculia colorata* Roxb. (Rajasekharreddy and Pathipati, 2014) and

Table 2
Effect of *Brachychiton populneus* extract on serum reduced glutathione content (GSH), malondialdehyde level (MDA) and nitric oxide content (NO) of Alloxan-induced diabetic rats.

Treatments	Parameters		
	GSH (mmol/l)	MDA (nmol/l)	NO (μmol/l)
Normal saline	2.44 ± 0.02	70.35 ± 1.05	2.47 ± 0.17
BPE	2.35 ± 0.03[b]	21.31 ± 1.88[b]	1.98 ± 0.15[b]
Alloxan	1.01 ± 0.12[a]	118.9 ± 5.29[a]	4.69 ± 0.48[a]

Values are expressed as mean ± SEM, n = 10.Values are statistically significant at $p < 0.05$.
[a] Significant from saline normoglycemic control.
[b] Significant from Alloxan hyperglycemic control.

B. populneus, while isovitexin was reported in *B. populneus*. Apigenin 6,8-di-*C*-glucoside was reported in *S. foetida* (Xia et al., 2009), *B. acerifolius* (Farag et al., 2015), *P. alata* (El Sherei et al., 2018) and *B. populneus*, while other reported di-*C*-glycosides of apigenin and luteolin were characteristic for *P. alata* (El Sherei et al., 2018). Finally, the 6-*C*-β-glucopyranoside-7-*O*-β-glucopyranoside of apigenin and luteolin were also characterized for *P. alata* (El Sherei et al., 2018).

A single isoflavone structure with a *C*-glucosyl substitution at position 8 (puerarin) had been reported in *S. foetida* (Xia et al., 2009). The only flavans and isoflavans were reported in *Hildegardia barteri* (Mast.) Kosterm. (Meragelman et al., 2005). Finally, anthocyanins were mainly reported as 3-*O*-glycoside derivatives of pelargonidin and cyanidin in *Sterculia parviflora* Roxb. and *Sterculia kunstleri* King. (Lowry, 1971), *B. acerifolius* (Farag et al., 2015).

Therefore, the variability of the flavonoid pattern of the six related genera (*Brachychiton*, *Firmiana*, *Hildegardia*, *Pterygota*, *Scaphium* and *Sterculia*) suggested that flavones, 6- or 8-hydroxyflavones, flavonols, isoflavones, flavans, isoflavans, *C*-glycosyl flavonoids and anthocyanins could be used as chemosystematic markers to differentiate between them (Table S1). These related genera are not only chemically different, but are also morphologically dissimilar. The macro-morphological characters displayed considerable variation in for example leaf (type, phyllotaxis, shape, size, apex, margin, base, venation, texture and petiole), inflorescence, fruit (dehiscence, surface, shape, size and color), as well as, seed (number, color, shape, surface and dimensions). The main micro-morphological characters differentiating the genera are the absence or presence of non-glandular and glandular trichomes, their features and the epidermal cells characters (Ragheb, 2017).

Biological investigation

Acute toxicity estimation of BPE

Acute lethal toxicity estimation of BPE; using the graphical method in mice (Lorke, 1983), showed 50% mortality of mice up to 7.211 g/kg with no adverse effects. Referring to conversion table of Paget and Barnes (1964), the LD_{50} of mice was altered to rat dose and calculated to be found 3.1 g/kg. Therefore, a dose of 500 mg/kg bw of the BPE was selected as an average dosing schedule for the biochemical studies.

Anti-hyperglycemic effect of BPE

Effect of BPE on BGL and BW. The effect of *i.p.* injection of the BPE (500 mg/kg) on blood glucose level (BGL) and body weight

Table 3
Flavonoid constituents of the five *Brachychiton* species growing cultivated in Egypt.

Compound	*B. populneus*	*B. acerifolius*	*B. rupestris*	*B. discolor*	*B. australis*
Flavones					
Apigenin	+[a]	+[c]	–	–	–
Apigenin 7-*O*-β-D-glucoside	+[a]	+[c]	–	–	–
Apigenin 7-*O*-β-D-glucuronide	+[a]	+[c]	–	–	–
Apigenin 7-*O*-(2"-α-rhamnoside)-β-glucuronide	–	+[c]	–	–	–
Luteolin	+[a]	+[c]	–	–	–
Luteolin 7-*O*-β-glucoside	+[a]	–	–	–	–
Luteolin 7-*O*-β-glucuronide	+[a]	+[c]	–	–	–
Flavonols					
Kaempferol	+[a,b]	+[c]	+[b]	–	–
Kaempferol 3-*O*-β-glucoside	+[a,b]	–	–	–	–
Kaempferol 3-*O*-rutinoside	+[a,b]	–	+[b]	–	
Kaempferol 3-*O*-(2",6"-dirhamnosyl)-β-glucoside [K 3-*O*-(2"-rhamnosylrutinoside)]	+[b]	–	+[b]	–	–
Kaempferol 3-*O*-(2",6"-dirhamnosyl)-β-galactoside [K 3-*O*-(2"-rhamnosylrobinoside)]	–	–	+[b]	–	–
Quercetin	+[a,b]	+[c]	+[b]	+[d]	+[e]
Quercetin 3-*O*-arabinoside	+[b]	–	–	–	–
Quercetin 3-*O*-rhamnoside (quercitrin)	+[a]	–	–	+[d]	–
Quercetin 3-*O*-β-glucoside	–	–	–	–	+[e]
Quercetin 3-*O*-galactoside (hyperoside)	–	+[c]	–	–	–
Quercetin 3-*O*-(6"-α-rhamnosyl)-β-glucoside (rutin)	+[a]	+[c]	–	–	+[e]
Quercetin 7-methyl ether (rhamnetin)	–	–	–	+[d]	–
Quercetin 3'-methyl ether (isorhamnetin)	+[a,b]	+[c]	+[b]	–	–
Isorhamnetin 3-*O*-rutinoside	+[a,b]	–	+[b]	–	+[e]
Isorhamnetin 3-*O* -(2",6"-dirhamnosyl)-β-D-galactoside [I 3-*O*-(2"-rhamnosylrobinoside)]	–	–	+[b]	–	–
C-Glycosylflavonoids					
Apigenin 6-*C*-β-glucopyranoside (isovitexin)	+[a]	–	–	–	–
Apigenin 8-*C*-β-glucoside (vitexin)	+[a]	–	–	–	–
Apigenin 6,8-di-*C*-β-glucoside(vicenin)	+[a]	+[c]	–	–	–
Anthocyanins					
Pelargonidin 3-*O*-glucoside	–	+[c]	–	–	–
Cyanidin 3-*O*-rutinoside	–	+[c]	–	–	–

(+): Present, (–): absent.
[a] Compounds isolated and detected in the present study.
[b] Compounds reported by Desoky and Youssef (1997).
[c] Farag et al. (2015); Abou Zeid et al. (2017).
[d] Kassem (2007).
[e] Kassem et al. (2002).

(BW) of alloxan-induced diabetic rats was determined at 0, 4 and 24 h time intervals. Results are reported as mg/dl (Table 1). At zero hour, no change in blood glucose level detected. After 4 h of treatment, BPE exhibited a decrease in blood glucose levels up to 227.3 ± 30.28 and throughout 24 h; a significant reduction was achieved (157.6 ± 16.57) at $p < 0.05$ compared to hyperglycemic control (488.33 mg/dl after 4 h and 448.50 mg/dl after 24 h). This result is comparable to that of the standard drug Diamicrone; 161.42 ± 11.15, after 24 h. Alloxan caused weight reduction by about 15.07%, which was nearly reserved by BPE (1.43%) at the end of 24 h. Diamicron restored the weight reduction by 9.97%.

Effect of BPE on the oxidative stress biomarkers (GSH, MDA and NO). Injection of BPE (500 mg/kg) to alloxan-induced diabetic rats, counteracted the effect of the oxidative stress induced by alloxan causing relative decrease in the MDA (21.31 nmol/l) and NO (1.98 μmol/l) levels and relative increase in the GSH level (2.35 mmol/l) in serum much more than the normal values (Table 2).

Treatment of diabetic rats with BPE elucidated a consistent acute anti-hyperglycemic and offered significant protection against oxidative stress and markedly improved antioxidant status of serum of rats with alloxan-induced diabetic rats after 24 h. This anti-hyperglycemic property could be ascertained from their antioxidant components. Flavonoids and phenolics elementary components are thought to prevent and manage the oxidative stress-related diseases (Engwa, 2018) through their functional hydroxyl groups which scavenge the free radicals and/or chelate the metal ions (Kumar et al., 2013). Thus, BPE may be effective in controlling hyperglycemia and oxidative damage and this activity could be attributed to their phenolic and flavonoid contents.

Conclusions

Seventeen flavonoids were isolated and identified, ten of which were reported for the first time from *B. populneus*. BPE (500 mg/kg bw) exhibited a significant antioxidant effect on the serum of alloxan-induced diabetic rats. BPE successfully restore the body weight of diabetic rats as well as controlling the levels of glucose in blood. The evaluated biological activities of BPE are believed to be related to the presence of chemical principles; flavonoids occurred in the species. Accordingly, further deep *in vivo* studies are necessary to develop those plants as potential chronic hypoglycemic phytoconstituents. From the chemosystematic point of view, the flavonoid profiles of the genus *Brachychiton* provide valuable taxonomic differences at interspecific and intergeneric levels.

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Conflicts of interest

The authors declare that we have no conflict of interest.

Authors'contributions

NAMS, MMES, SAM, MESK and MMM created the point, supervised the study, the writing and review of the manuscript. AYR collected the plant material, worked on laboratorial work. MESK and MMM assisted in carrying out the laboratory work. AYR and MMM performed the microscopic analysis, wrote and formatted the manuscript. All the authors have read and review the final manuscript and approved the submission.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2019.05.001.

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