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Abstract

The first reported phytochemical studies on two species of flowers in Australia enabled the identification of six secondary metabolites from Illawarra flame tree flower (Brachychiton acerifolius) and seven secondary metabolites from the flowers of the Alstonville (Tibouchina lepidota). Pelargonidin 3-(6-coumarylglucoside)-5-(6-acetylglucoside) was found to be responsible for the red colour of B. acerifolius, whereas malvidin 3-(coumarylglucoside)-5-(acetylxyloside) was responsible for the purple colour of (T. lepidota) flowers. (2S)-4,5-Dihydroxyflavanone 7-O-β-d-glucuronide methyl ester was isolated for the first time from B. acerifolius, and its absolute configuration was determined by circular dichroism spectroscopy. Some of the traditional uses of B. acerifolius could also be correlated with the known activity of the isolated metabolites.

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Flowers in Australia: Phytochemical Studies on the Illawarra Flame Tree and Alstonville

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Abstract

The first reported phytochemical studies into two species of flowers in Australia resulted in the identification of six secondary metabolites from Illawarra flame tree flower (*Brachychiton acerifolius*) and seven from the flowers of the Alstonville (*Tibouchina lepidota*). Pelargonidin 3-(6-coumarylglucoside)-5-(6-acetyl-glucoside) was found to be responsible for the red colour from *B. acerifolius* while malvidin 3-(coumarylglucoside)-5-(acetylxyloside) for the purple colour from (*T. lepidota*) flowers. (2*S*)-4,5-Dihydroxylavanone 7-*O*-β-D-glucuronide methyl ester was isolated for the first time from *B acerifolius* and its absolute configuration was determined by circular dichroism spectroscopy. Some of the traditional uses of *B. aerifoius* could also be correlated to the known activity of the isolated metabolites.

Before European settlement of Australia, the indigenous people used plants and flowers for numerous purposes including as nutrients, medicine, and dyes.[1] Australia has over 30,000 described species of vascular plants which grow in a wide range of climatic types encompassed by the continent allowing a diversity of plants and flowers unlike any other country.[2] However, there is limited information available pertaining to the phytochemical studies of Australian flowers.

The Illawarra flame tree (*Brachychiton acerifolius*), a member of the Malvaceae family, is widespread in the subtropical rainforests on the East coast of Australia, stretching north from the Shoalhaven River (NSW) up to Queensland.[3] Together with other members of the genus Brachychiton, it is commonly referred to as a Kurrajong. For the indigenous people, the Kurrajong seeds are used both as a nutrients and as an emollient with antiseptic properties.[1] It has been reported that the genus of Brachychiton possess biological activities including antimicrobial, anticancer[4], and anti-diabetic.[5] A cursory study of the methanol extract from the Illawarra flame tree flowers reported moderate antimicrobial activity against *Bacillus cereus*[6] whereas, the antioxidant activity from the leaves showed high activity (IC_{50}: 0.015 mg/mL) compared to Vitamin C as a positive control.[7] Despite these reports, there is no information available on the chemical structures contained within this extract.

Alstonville (*Tibouchina lepidota*) is a shrub belonging to the family Melastomataceae and is a native of South America. The genus of Tibouchina is predominantly distributed in tropical and subtropical regions of the Americas and includes about ca. 350 species.[8] *T. lepidota* was introduced during 1978 at Alstonville, NSW and it became popular in New South Wales and Queensland (Australia), bearing beautiful dark purple flowers in late summer through to autumn.[9] The genus of Tibouchina has been used as traditional medicine in Brazil, e.g. an infusion of *T. grandifolia* Cogn. leaves has been used for its wound healing properties,[10] and isolated compounds from this genus show antibacterial[11], antioxidant[12], and anti-parasitic[13] activities.

Despite their bright colourful flowers and the reported biological activities of extracts, there are only limited phytochemical studies and no reports on the structures contained within both *Brachychiton acerifolius* and *Tibouchina lepidota*. In this report, we present details on the extraction, isolation and identification of compounds from both species, which then enabled the identification of the compounds responsible for the intense colouration present in the flowers, as well as being able to correlate known tradition medicinal uses to the biological activities of some isolated compounds.

Freeze-dried samples of flowers from both species of plants were extracted with methanol and solid-liquid back extracted with hexane to remove the non-polar compounds. The presence of secondary metabolites in the hexane extract was examined using GC-EIMS (data in supplementary material). The polar extract of the flowers of both species were subjected to analytical HPLC followed by semi-preparative reverse-phase HPLC to reveal 13 secondary metabolites. Six compounds were isolated from the Illawarra flame tree flower (*B. acerifolius*): these were quercetin **1**[14], pelargonidin 3-(6-coumarylglucoside)-5-(6-acetylglucoside) **2**[15], kaempferol 3-rutinoside **3**[14], and kaempferol **6**[14]. Compound **4** and **5** were isolated as naringenin 7-*O*-β-glucuronide and 4,5-dihydroxylavanone 7-*O*-β-D-glucuronide methyl ester and their NMR spectroscopic data and low-resolution electron impact mass spectrometry were compared (see supporting information).[16] The absolute configuration of both compounds were assigned using optical rotation and CD spectroscopy, with the CD spectrum of **5** showing cotton effects (248 (Δε +0.59), 287 (Δε -1.60) nm). This indicated that the absolute configuration at the C2-position of **5** is in an (*S*)-orientation, opposite to the compound previously reported.[16] Therefore, we report here for the first time the isolation and characterization of the (2*S*)-4,5-dihydroxylavanone 7-*O*-β-D-glucuronide methyl ester.

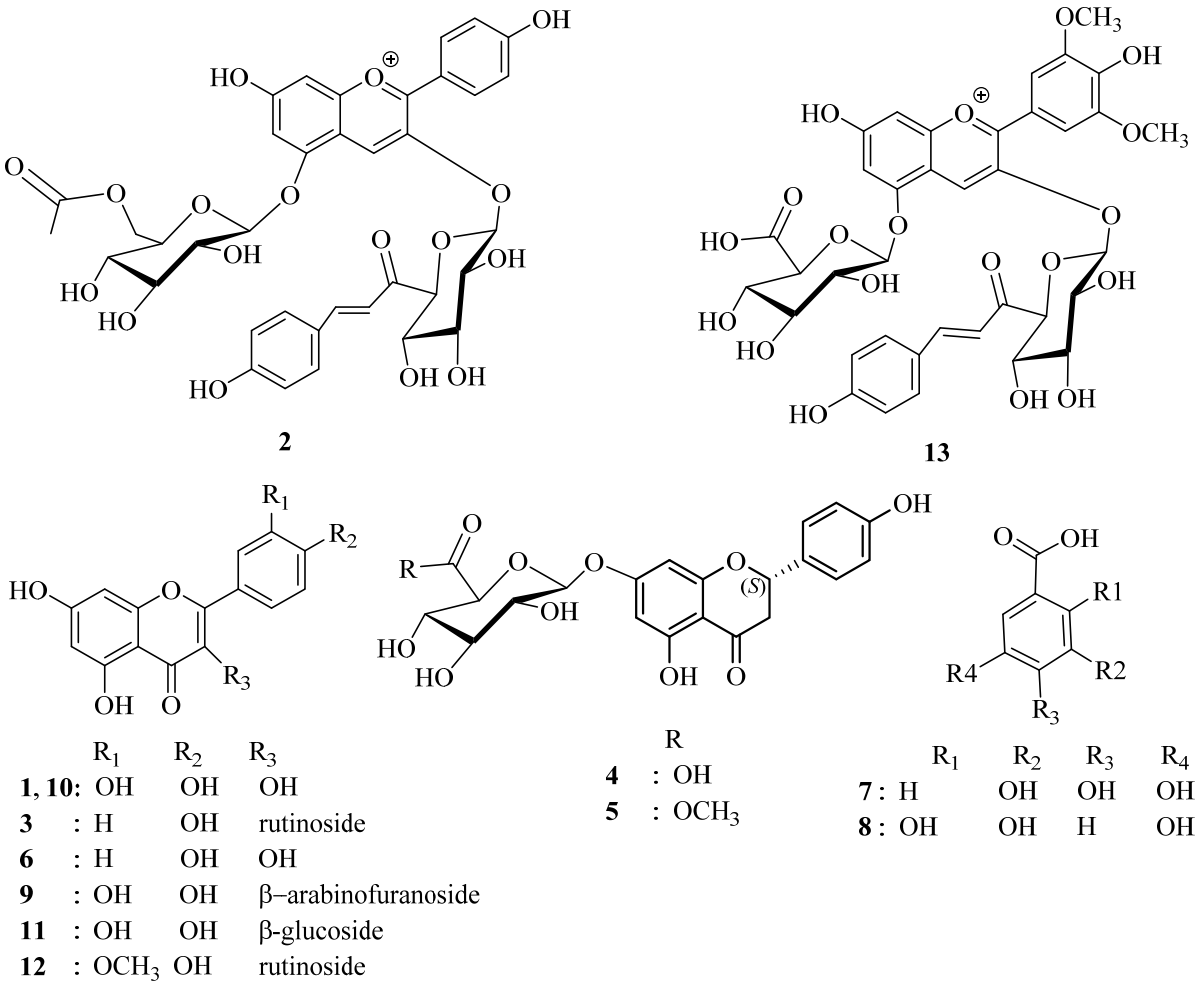

Figure 1. Structures of secondary metabolites from the Illawarra flame (*B. acerifolius*) and Alstonville flowers (*T. lepidota*).

In addition, gallic acid **7**[17], 2,3,5-trihydroxybenzoic acid **8** [17, 18], avicularin **9**[12], quercetin **10**[12], quercetin 3-glucoside **11**[14], isorhamnetin 3-rutinoside **12**[19], and malvidin 3-(coumaryl-glucoside)-5-(acetylxyloside) **13**[20] were isolated from Alstonville (*T. lepidota*) flower. All the isolated secondary metabolites have been previously reported and their spectroscopy data were compared as part of this study but importantly, they were isolated from these species here for the first time. Compound **2**, a red solid and **13**, a purple solid were

identified as the anthocyanins responsible for the red colour of the *B. acerifolius* flowers and the purple colour of the *T. lepidota* flowers respectively. Both compounds contain acetic acid as an acylglucosyl moiety and this type of acetylated anthocyanin has been reported in a number of plants such as *Eurya japonica, Verbena hybrid* and *Tibouchina urvilleana*.[15]

Other members of the genus Brachychiton have been used as source of nutrition, e.g. the *Brachychiton diversifolius* seed and additionally *B. discolor* and *B. acerifolius* are reported to possess high protein content, and essential amino acids, indicating their potential as feed supplements.[21] Sourcing the Australian Aboriginal pharmacopoeia, the seed of *B. acerifolius* is used as an emollient for its antiseptic properties[1] as well as for a food source but until now, there has been no laboratory studies to support this claim. The antibacterial activities of the crude methanol extracts from the leaves and flowers of *B. acerifolius* have been reported against several pathogenic bacteria with the flowers possessing high antibacterial activity compared to the leaves against *Bacillus cereus*.[6] This is likely due to the presence of kaempferol and quercetin which have been widely reported to have antibacterial activity against *Bacillus sp*. and other human pathogenic bacteria.[22, 23]

Alstonville (*T. lepidota*) is used mostly as an ornamental plant due to their dark purple flowers with no medicinal usage previously reported. However, other species within the genus have been reported to possess various biological activities such as wound healing and to treat cataract.[24] The isolated compounds **9** – **11** from our study were found to be present in *T. semidecandra* L. leaves[12] which possess antioxidant and anti-tyrosinase activities.[12] Furthermore, published data showed that compounds **7** and **8** possess antitumor[17] activities whereas compound **7** was believed to possess high antioxidant activity[17] and plays an important role in the prevention of malignant transformation and cancer development.[25] *T. grandifolia* Cogn is used as wound healing properties in Brazil and this is likely due to the presence of quercetin 3-glucoside (**11**) and is reported to have *in vivo* wound healing properties via linear incision and circular excision wound models.[26] It is evident that the flowers of *T. lepidota* serve not only an ornamental purpose, but also as medicinal plant.

In summary, we report here the first phytochemical studies on *B. acerifolius* and *T. lepidota* flowers, revealing the presence of compounds **1-13** which can account for the antimicrobial, antiseptic activities which previously cited in the Australian Aboriginal pharmacopoeia.[1] Further, we report here for the first time the isolation and characterization of 2*S*)-4,5-dihydroxylavanone 7-*O*-β-D-glucuronide methyl ester. The anthocyanins (**2**, **13**) from both species are not only responsible for the colouration of these flowers but also possess antioxidant, antimicrobial and anticancer activities.[27]

Experimental

Plant Material

The Illawarra flame (*B. acerifolius*) flowers were collected in October 2013 from the campus grounds of the University of Wollongong and Alstonville (*T. lepidota*) flowers were collected in April 2013 from Aschroft Place, Wollongong, NSW. All the flowers were washed, freeze-dried, and stored in refrigerator until analysis.

Extraction and Isolation

The freeze-dried flowers (300 g) were crushed, suspended in methanol (1.0 L) and stirred for 24 h, then filtered, and the filtrate then extracted with methanol (3 x 1 L). The supernatants were pooled and concentrate *in vacuo* to produce 13.538 g (Illawarra flame) and 14.346 g (Alstonville). The extracts solutions were back-extract with hexane (1.0 L), dichloromethane (1.0 L). The polar extract (3.174 g and 4.328 g, repectively) was re-dissolved in methanol (10 mL) and filtered through a HPLC filter (0.45 μm). The extract was chromatographed by semi-preparative HPLC with solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in ACN) as eluent developed in gradient from 90% to 55% solvent A within 40 to 50 minutes.

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