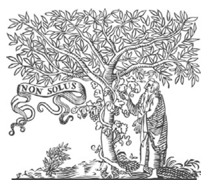

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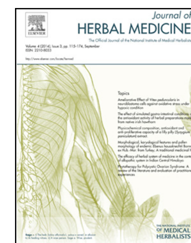

Original Research Article

Physicochemical composition, antioxidant and anti-proliferative capacity of a lilly pilly (*Syzygium paniculatum*) extract

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ABSTRACT

Lilly pilly (LP) fruit (*Syzygium paniculatum* Gaertn.) is widely grown in eastern Australia and has been used as food by indigenous Australians. However, there is limited information on its bioactivity. This study investigated the physicochemical and antioxidant properties of the crude fruit extract, identified its bioactive compounds and also assessed its potential anti-proliferative effect on pancreatic cancer cells. Our data showed that the LP extract was water-soluble and possessed a total phenolic content of 96 mg of gallic acid equivalents (GAE)/g, flavonoid levels of 52 mg catechin equivalents (CAE)/g, proanthocyanidin levels of 29 mg CAE/g. Several phenolic compounds such as gallic acid, chlorogenic acid, catechin and epicatechin were identified in the LP extract with levels of 0.39, 2.35, 0.47 and 2.9 mg/g, respectively. Results from six different antioxidant assays revealed that the LP extract pocessed potent antioxidant and free radical scavenging capacity. Although antioxidant capacity of the extract was lower than that of vitamin E, vitamin C and BHT, it could be significantly improved if the extract was to be further purified. We also showed that the LP extract (200 μg/mL) significantly reduced the viability of MiaPaCa-2 and ASPC-1 pancreatic cancer cells to levels comparable to that of the chemotherapeutic agent gemcitabine. For this reason lilly pilly should be further investigated for its health promoting and potential anti-cancer benefits, particularly for pancreatic cancer.

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1. Introduction

The magenta lilly pilly (LP; *Syzygium paniculatum Gaertn.*) belongs to the family Myrtaceae and is a small to medium-sized rainforest tree endemic to coastal New South Wales (NSW), Australia (Hyland, 1983). The LP produces fruit in the form of purple berries (15–25 mm diameter) between March and May. The fruit is shiny in appearance and possesses fleshy distal calyx lobes (Floyd, 2008). It has a pleasantly sour apple-like flavour and can be eaten fresh or made into jams (Floyd, 2008). Lilly pilly is similar to other Australian flora in that it has developed unique survival characteristics to adapt to diversified environmental and climatic conditions (Mohanty and Cock, 2012); as such, the LP fruit may contain key bioactive components which may provide enhanced health benefits such as anti-cancer activity in humans. While LP has been a staple in the diet of indigenous Australian communities for many years, only limited information on the chemical profile of the fruit exists. Quijano-Célis et al., (2013) analysed volatile constituents using gas chromatography; reporting a total of 155 individual compounds, with terpenes (α-pinene – 32.8%, (Z)-β-ocimene – 21.8%, limonene – 6.9% and α-terpinol 5.1%) dominating the volatile oil profile. A second study by Longo et al. (2007) analysed the anthocyanin composition, identifying only one compound – malvidin 3,5-diglucoside (325.9 mg/kg). To the best of our knowledge, this study is the first report profiling the chemical, antioxidant and biological properties of the LP fruit.

Polyphenols and their secondary metabolites such as flavonoids and proanthocyanidins have frequently been identified as the bioactive constituents in fruits possessing potent antioxidant and anti-cancer properties (Pierson et al., 2012; Rossi et al., 2010, 2012). Plant derived substances (phytochemicals) are the richest source of medicines for use in traditional and Western medicines, offering potential in direct treatment and in synthetic drug development (Tiwari et al., 2011). The extraction and assessment of plant phytochemicals is therefore essential to the ongoing development of new and novel disease therapeutics. In particular, with pancreatic cancer considered to be one of the most devastating human cancers, due to its difficulty of diagnosis and limitations in effective treatment options (Scarlett et al., 2011), novel therapeutic options need to be explored. Consequently, the aim of this study was to investigate the physicochemical properties of the magenta lilly pilly and identify bioactive components within lilly pilly extract. The antioxidant capacity and anti-proliferative effect on pancreatic cancer cells were also established.

2. Materials and methods

2.1. Materials

Ripe LP fruit was harvested in March, 2013 from cultivated plants located at Ourimbah, Central Coast, NSW, Australia (latitude of 33.4° S, longitude of 151.4° E). The plants were authenticated by one of the authors (A.C.C.) and a voucher specimen deposited at the Don McNair Herbarium, the University of Newcastle, NSW, Australia. Once harvested, the fruit was returned to our laboratories where it was immediately frozen in liquid nitrogen and freeze dried (FD3 freeze dryer, Thomas Australia Pty. Ltd., Seven Hills, NSW, Australia). Using a blender (John Morris Scientific, Chatswood, NSW, Australia), the dried fruit was then ground and the resulting powder sieved (≤2.8 mm) using a steel mesh sieve (2.8 mm EFL 2000; Endecotts Ltd., London, England) before being packed in a sealed container and stored at 5 °C until required.

Table 1 – Bioactive properties of the crude lilly pilly extract.

Properties	Values
Total phenolic compounds (mg GAE/g)	96.58 ± 3.95
Flavonoids (mg CAE/g)	52.50 ± 3.33
Proanthocyanidins (mg CAE/g)	29.25 ± 2.42
Monomeric anthocyanins (mg CGE/g)	2.43 ± 0.20
Vitamin C (mg AAE/g)	174.12 ± 6.95
Gallic acid (mg/g)	0.39 ± 0.01
Chlorogenic acid (mg/g)	2.35 ± 0.05
Catechin (mg/g)	0.47 ± 0.01
Epicatechin (mg/g)	2.9 ± 0.04

2.2. Extraction and preparation of the crude LP extract

Powdered LP fruit (25 g) was extracted in 80% (v/v) ethanol (500 mL) at room temperature (RT) for 48 h using a stirrer at 600 rpm. The extract was then filtered using Whatman No. 1 filter paper (Lomb Scientific, Taren Point, NSW, Australia) and concentrated using a rotary evaporator (Buchi Rotavapor B-480, Buchi Australia, Noble Park, Vic., Australia) at 40 °C under pressure. The concentrated extract was then frozen using liquid nitrogen and freeze dried to yield the crude LP extract which was stored at −18 °C until required.

2.3. Determination of physical properties of the crude LP extract

Lilly pilly dry matter and moisture content, solubility, pH and colour were determined using previously described methods (Vuong et al., 2012, 2013) with minor modifications. Briefly, the LP extract (1 g) was dissolved in water (100 mL) using a magnetic stirrer (600 rpm, RT for 5 min) and then centrifuged (3000 × *g*, 5 min). The pH and colour of the supernatant was then measured (Table 1). 10 mL of the supernatant was vacuum dried at 70 °C to a constant weight then solubility determined as the weight of dryness (g) in 100 mL × 100/g of extract.

2.4. Determination of chemical composition of the crude LP extract

40 mg of crude LP extract was diluted in 100 mL of methanol and its chemical composition assessed. Total phenolic content (TPC), flavonoid and proanthocyanidin concentrations were determined as described by Vuong et al. (2013) and expressed as mg of gallic acid equivalents (mg GAE), mg of catechin equivalents (mg CAE), per gram of the LP extract, respectively. Total monomeric anthocyanin pigment was measured as described by Lee et al. (2005) and the results expressed as

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