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Ali Vafaei, Jamaludin Bin Mohamad & Ehsan Karimi

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HPLC profiling of phenolics and flavonoids of *Adonidia merrillii* fruits and their antioxidant and cytotoxic properties

Ali Vafaeia, Jamaludin Bin Mohamadb and Ehsan Karimib

aFaculty of Science, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia; bDepartment of Biology, Islamic Azad University, Mashhad, Iran

**ABSTRACT**

In this study the antioxidant and cytotoxicity activity of the *Adonidia merrillii* fruits were investigated using different solvent polarities (methanol, ethyl acetate and water). The results showed that the total phenolic and flavonoid contents of the methanolic extract was higher compare with other extract with respective values of 17.80 ± 0.45 mg gallic acid equivalents/g dry weight (DW) and 5.43 ± 0.33 mg rutin equivalents/g DW. Beside that The RP-HPLC analyses indicated the presence of gallic acid, pyrogallol, caffeic acid, vanillic acid, syringic acid, naringin and rutin. In the DPPH, NO2 and ABTS scavenging assays, the methanolic extract exhibited higher antioxidant activity as compared to the ethyl acetate and water extracts. The extracts exhibited moderate to weak cytotoxic activity in the assays using human hepatocytes (Chang liver cells) and NIH/3T3 (fibroblasts cell) cell lines. The findings showed the *Adonidia merrillii* fruit extracts to possess considerable antioxidant and cytotoxicity properties. The fruit, therefore, is a potential candidate for further work to discover antioxidant and cytotoxic drugs from natural sources.
1. Introduction

In recent years, there has been growing interest in the application of bioactive plant secondary metabolites as medicinal agents since synthetic drugs had shown several side effects on the human body (Manian et al. 2008). Recent studies revealed the role of phenolic and flavonoid compounds as major secondary metabolites implicating biological activities of plant extracts (Ao et al. 2008). They have also been shown to be effective inhibitors of platelet aggregation (Narayana et al. 2001). *Adonidia merrillii* of Arecaceae family is commonly known as the ‘Christmas Palm’ because of its fruits becoming bright scarlet and tends to be that color in winter. In order to hasten the development of biopharmaceutical aspects of *Adonidia merrillii* fruits, it is pertinent to establish the scientific knowledge of its medicinal properties as little is known on the chemical constituents of this plant. There is no research on antioxidant and cytotoxicity of *Adonidia merrillii* has been reported. The aim of this study was to evaluate the antioxidant and cytotoxicity activity of the *Adonidia merrillii* fruits.

2. Results and discussion

Significant differences ($p < 0.05$) in phenolic content of methanolic, ethyl acetate and water extracts of *Adonidia merrillii* fruits were observed, with values of $17.8 \pm 0.37$ and $2.22 \pm 0.25$ mg GAE/g DW, respectively (Table S1). Similarly, the flavonoid contents were markedly higher in the methanolic extract, with a value of $5.43 \pm 0.33$ mg rutin equivalent/g DW compared to the ethyl acetate extract at $3.12 \pm 0.54$ mg and the water extract with a value of $1.10 \pm 0.63$ mg rutin equivalent/g DW. Our result with *Adonidia merrillii* fruit extracts are in agreement with that of Chang et al. (1993), who also reported that methanol was the most effective solvent to extract bioactive compounds. Methanol has been reported as an efficient solvent to extract phenolic and flavonoid compounds (Do et al. 2014). The results of the Reversed-phase (RP) chromatography analysis clearly showed that methanolic extracts exhibited higher levels of phenolics (Table S2) and flavonoid compounds (Table S3) compared to the water and ethyl acetate extracts. The methanolic extracts of *Adonidia merrillii* fruits demonstrated that pyrogallol and gallic acid were the major phenolic compounds with values of $913.11 \pm 0.79$ and $101.61 \pm 0.19$ μg/g dry weight, respectively. The results of the methanolic extracts also showed that naringenin was the main flavonoid compound in *Adonidia merrillii* fruits with a mean value of $895.41 \pm 0.36$ μg/g dry sample. Compounds such as pyrogallol, gallic acid, naringin and quercetin have been reported to possess antioxidant properties as well as anti-inflammatory activities (Manpong et al. 2009; Nicolis et al. 2008). Free radical scavenging activity (DPPH) of the methanolic *Adonidia merrillii* fruit extract was stronger than that of the ethyl acetate extract, and was followed by the water extract (Figure S1A). All the values, however, were lower than those obtained for Vitamin E, BHT and Vitamin C that were used as antioxidant standards. The $\text{IC}_{50}$ (required concentration to inhibit 50% of DPPH radicals) of Vitamin E, Vitamin C and BHT were found to be $1.47 \pm 28.8$, $3.04 \pm 25.1$ and $31.31 \pm 2.43$ μg/mL, respectively (Table S4). The overall results demonstrate the antioxidant activities of methanol, ethyl acetate and water extracts as well as the standards in reactions with nitric oxide (NO) (Figure S1B). The extracts inhibited the NO in a dose dependent manner. The $\text{IC}_{50}$ concentrations showed significant ($p < 0.01$) differences in NO scavenging activity among samples (Table S4), where the methanolic extract showed the lowest value followed by ethyl acetate and water extracts, respectively. Ethyl acetate and
water extracts did not show ABTS radical cation-scavenging activity, whereas methanolic extracts exhibited 51.49% radical scavenging activity at 1 mg/mL (Figure S1C). The IC$_{50}$ concentrations also showed that ABTS scavenging activity of the methanolic extract was stronger than ethyl acetate and water extracts (Table S4). Antioxidants are responsible for preventing oxidative damage to cellular components as a consequence of biochemical reactions. Some phenolics and flavonoids appeared to be more active than vitamins for this purpose and their activities depend on the structure and total number of hydroxyl groups (Oskoueian et al. 2011). The radical scavenging activity of Adonidia merrillii fruit extracts could be attributed to the different types of bioactive compounds such as phenolic and flavonoid compound. Rapisarda et al. (1999) reported the antioxidant capacity of some varieties of pigmented oranges including Moro, Sanguinella, Tarocco and Washington. All examined orange juices showed antioxidant capacity, due to total phenolic contents and to their ability to interact with the bio-membrane; their antioxidant capacity seems to be widely influenced by the anthocyanin concentration in the pigmented orange juices. This radical scavenging activity of extracts could be related to the nature of the phenolic and flavonoid compounds that are contributing to their electron transfer/hydrogen donating ability (Loganayaki et al. 2013). The results on cytotoxic activity of methanolic (Figure S2A,D), ethyl acetate (Figure S2B,E) and water extract (Figure S2C,F), ethyl acetate and water extracts of Adonidia merrillii fruits show that increase in extract concentration of up to 1000 μg/mL, could reduce the cell viabilities significantly ($p < 0.01$) in a dose-dependent manner in the two cell lines tested. The results showed that all the extracts could inhibit Human hepatocytes (Chang liver cells) and NIH/3T3 (Fibroblasts cell). The methanolic extract was more active against Chang liver cell and NIH/3T3 compared to the ethyl acetate and water extracts (Table S5). The study on in vitro cytotoxicity against NIH/3T3 (Fibroblasts cells) and Human hepatocytes (Chang liver cells) indicated that there was a weak to moderate cytotoxic activity exhibited by the different extracts and that the compounds which were present in the extracts were non-toxic to humans. This suggests that these compounds can be considered as suitable agents as potential therapeutics. According to Boyd (1995) a plant extract is usually regarded as interesting for in vitro cytotoxic activity when IC$_{50}$ < 100 μg/mL. In the present study, all the extracts showed interesting potential for in vitro cytotoxic activity to all cells with various IC$_{50}$. Jonville et al. (2008) mentioned that the definition of promising activity was reserved for extracts with IC$_{50}$ values of less than 50 μg/mL and that further investigations with regard to isolation of compounds and drug mechanisms were needed. Previous studies have demonstrated that isolated polyphenols and various flavonoid compounds from different plants have notable anticancer activities against different cell lines (Ramos 2007; Mavundza et al. 2010). For instance Manthey et al. (2001) reported that citrus flavonoids inhibited the growth of HL–60 leukemia cells. Luo et al. (2009) showed that kaempferol inhibited the growth of ovarian cancer cell lines (91%), and A2780/CP70 (94%) at concentration of 20 and 40 μM, respectively. Quercetin has been shown to inhibit prostate cancer growth (Verschoyle et al. 2007).

3. Conclusions

This preliminary study indicated the pyrogallol and naringenin were the main phenolic and flavonoid compounds in in the various extracts of Adonidia merrillii fruits respectively. Meanwhile, the methanolic extract showed appreciable antioxidant and anticancer properties compared to other extracts. The results from this study suggests the Adonidia merrillii
fruits as novel potential sources of natural antioxidants in the future. The present study provides novel scientific data on *Adonidia merrillii* fruits, including information on flavonoid and phenolic compounds which has never been reported in previous investigation.

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**Disclosure statement**

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