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Angiotensin-Converting Enzyme Inhibitory Action of Selected Plants

Supplementary Material

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Bixa orellana (Bixaceae), commonly known as annatto, is locally referred to as *atsuete*. The methanol extracts from the leaves and seeds of annatto exhibited anti-bacterial activity (Medina-Flores et al. 2016). The methanol leaf extract of annatto showed neuropharmacological, anticonvulsant, analgesic, and antidiarrhoeal activities in mice (Shilpi et al. 2006). The bark extract rendered protective anti-oxidant activity in acetaminophen-induced hepatic damage in rats (Bell et al. 2012).

Artocarpus heterophyllus (Moraceae),commonly known as jack fruit, is locally referred to as *langka*. The polysaccharide isolated from its pulp has a strong anti-oxidant activity (Zhu et al. 2017). The ethanol extract from its stem bark inhibited the action of a-amylase and a-glucosidase, an activity with potential anti-diabetes applications (Ajiboye et al. 2016). Preclinical studies have shown that jackfruit possesses several bioactivities including anti-inflammatory, antibacterial, anticariogenic, antifungal, antineoplastic, and wound healing effects (Baliga et al. 2011).New phenolic compounds have also been isolated from its leaves (Wang et al. 2017).

Morus alba (Moraceae), commonly known as white mulberry, is locally referred to as *moras*. Flavonoids and cinnamic acids were identified from the ethanol extracts obtained from its leaves. The same extract showed a high degree of activity against inflammation but was found to be toxic in mice (de Oliveira et al. 2016). Its root bark has shown therapeutic potential against diabetes-induced depression in mice (Ye et al. 2017).Compounds isolated from its root bark exhibited potential anti-obesity activity by inhibiting the action of pancreatic lipase (Ha et al. 2016). New glycoside and flavones were also isolated from its stem bark (Ali and Ali 2016).

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Nymphaea pubescens or water lily is part of the Nymphaeacea family. Its aqueous extracts exhibited anti-inflammatory and hepatoprotective effects in rats (Debnath et al. 2013).

*Syzygium samarangense (*Myrtaceae), commonly known as water apple, is locally referred to as *makopa*. The pulp and seeds contain cytotoxic chalcones and anti-oxidant glycosides (Simirgiotis et al. 2008). Vescalagin isolated from its fruit exhibited therapeutic value against diabetes through its anti-hypertriglyceridemic and anti-hyperglycemic effects (Shen and Chang 2013). Aurentiacin from the said plant displayed anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated mouse macrophages (Kim et al. 2012).

EXTRACTION

The leaves of *B. orellana, A. heterophyllus, M. alba, N. pubescens* and *S. samarangense* were collected within the University of the Philippines Diliman campus and submitted to the Dr. Jose Vera Santos Herbarium, Institute of Biology, UP Diliman for verification. The accession number of *B. orellana* is 3649, *A. heterophyllus* is 9431, *M. alba* is 14626, *N. pubescens* is 3498, and *S. samarangense* is 14258.

The leaves were washed and air dried. The dried leaves were macerated using a blender and soaked in methanol. The solution was filtered to obtain the crude organic extract. The crude methanol extract was partitioned between distilled water and hexane in a 1:2 ratio, followed by the partitioning of the remaining aqueous layer with ethyl acetate using the same ratio. The hexane and ethyl acetate partitions were then concentrated *in vacuo*.

PHYTOCHEMICAL SCREENING

The phytochemical screening protocol was modified from the works of Harborne (1984), Edeoga et al. (2005), and Onwukaeme et al. (2007). The qualitative test for the presence of saponins, flavonoids, tannins, cardiac glycosides, phenolic compounds, alkaloids, and terpenoids were performed on 20 mg of the methanol leaf extracts dissolved in 200 µL DMSO.

ANGIOTENSIN CONVERTING ENZYME INHIBITORY ASSAY

The method for the determination of the ACE inhibition of the plant extracts was modified from the procedure of Jimsheena and Gowda (2009). A stock solution was

prepared using one mg of the sample dissolved in 20 µL methanol. The mixture was diluted to 0.5 mL using the 0.05M sodium borate buffer. Thirty five µL of the stock solution was mixed with an equal volume of the sodium borate buffer in a microplate. A positive control solution was made using 35µL of captopril as the ACE inhibitor. A blank solution containing twice the volume of the buffer solution was also prepared. The reaction was initiated by the addition of 10 µL of ACE in each solution. The plate was shaken for 15 seconds and incubated at 37°C for 10 minutes. Twenty µL of the hippuryl-histidyl-leucine (HHL) substrate was added to the solutions. The plate was shaken for an additional 15 seconds and incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 50 µL hydrochloric acid. One hundred µL pyridine and 50 µL benzene sulfonyl chloride (BSC) were added to the solutions to produce a change in color. The absorbance of each solution was measured at 410nm. All measurements were performed in triplicates.

Using the absorbance measurements of the solutions, the extent of inhibition was calculated using the following formula:

$$\text{ACE inhibitory activity } (\%) = \frac{B - A}{B - C} \times 100, \qquad (1)$$

where *B* is the absorbance of control (buffer was added instead of the test sample), *C* the absorbance of the reaction blank, and *A* isthe absorbance in the presence of the sample.

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