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Ipomea aquatica CRUDE EXTRACT INHIBITS LIPOXYGENASE, HYALURONIDASE AND XANTHINE OXIDASE ACTIVITIES

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Abstract

Ipomea aquatica, locally known as water spinach, is one of the most common vegetable consumed by Malaysian. Based on previous studies, crude extract and phenolic compounds of *I. aquatica* exhibited several biological activities including antioxidant, anti-microbial and anti-proliferative. The presence of phenolic compounds in *I. aquatica* may contributed to their ability to inhibit enzymes, chelate metals and scavenge free radicals. Currently, no study reported on anti-inflammatory activity of *I. aquatica* with respect to lipoxygenase, hyaluronidase and xanthine oxidase enzymes. The present study aims to enhance current knowledge on biological properties of *I. aquatica* crude extract particularly on anti-inflammatory activity. Three enzymes that involve in inflammatory pathway were selected in this study including lipoxygenase, hyaluronidase and xanthine oxidase. *I. aquatica* was extracted in methanol and tested for lipoxygenase, hyaluronidase and xanthine oxidase at different concentrations using direct enzyme inhibition assay. Lipoxygenase, hyaluronidase and xanthine oxidase inhibitory activities of the methanol crude extract increased with increasing concentration. Highest inhibition activity against lipoxygenase, hyaluronidase and xanthine oxidase were observed at a concentration of 1000 µg/ml with inhibition of 87.18%, 95.36% and 78.38%, respectively. Our finding in this study indicates potential anti-inflammatory activity of *I. aquatica* crude extract through inhibition of lipoxygenase, hyaluronidase and xanthine oxidase.

Keywords: *Ipomea aquatica***,** lipoxygenase, hyaluronidase, xanthine oxidase, anti-inflammatory

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Introduction

Ipomea aquatica belongs to Convolvulaceae family that widely distributed throughout tropical and subtropical region. In Malaysia, this plant is known as water spinach and one of the most common vegetables consumed by Malaysian (Izzah et al., 2012) due to its easy cultivation and wide availability (Kitayama et al., 2019). The leaf of *I. aquatica* contains vitamin C, vitamin E and riboflavin (Prasad et al., 2008). Recent study showed that the stem of *I. aquatica* contains higher amount of crude protein and fibre than leaf and petiole (Shariff et al., 2019). Chemicals profiling of *I. aquatica* extracts showed the presence of polyphenols including quercetin, apigenin and luteolin (Prasad et al., 2005). This plant has been used in traditional medicines to treat constipation (Samuelsson et al., 1992), abscess (Ong et al., 2011), diabetes (Malalavidhane et al., 2003) and liver malfunction (Badruzzaman & Husain, 1992).

Crude extracts and isolated compounds from this plant revealed several biological properties such as anti-hyperglycemic activity (Malalavidhane et al., 2001 and Sajak et al., 2017) and hepatoprotective effects in rats (Alkiyumi et al., 2012), proliferation inhibition against HepG2 cells (Fan et al., 2014), and direct acetylcholinesterase activity inhibition (Dhanasekaran et al., 2015). Study on violaxanthin, a carotenoid from *I. aquatica*, showed stronger free radical scavenging activity than β-carotene and

lutein. In addition, violaxanthin also able to inhibit hemolysis of blood cell and lipid peroxidation in mouse model (Fu et al., 2011). *I. aquatica* in aqueous extract inhibited lead-intoxication in murine hepatocytes cells through enhancement of antioxidant enzyme and glutathione, reduced reactive oxygen species, lipid peroxidation and carbonylation of protein. In addition, *in vivo* study also indicated that *I. aquatica* reduced accumulation of lead in mice organs including kidney, liver, heart, testes and brain (Dewanjee et al., 2015).

Inflammation is a body's defence mechanism which involve immune system against injury, microbial infection and irritation (Clark & Kupper, 2005). Inflammation is beneficial, self-limiting and rapid. However, chronic inflammation, characterize by prolonged and slow inflammation, can cause injury to host tissue. This condition involves activation of various inflammatory-related enzymes. Chronic inflammation has been linked to metabolic disorder, neurological and cardiovascular diseases (Libby, 2007). Thus, inhibiting inflammatory-related enzymes may help in reducing severity of these diseases. Currently, no study reported on inhibition of inflammatory enzymes such as xanthine oxidase, lipoxygenase and hyaluronidase enzyme by *I. aquatica*. Therefore, the present study investigated inhibitory effect of *I. aquatica* extract on inflammatory-related enzymes using direct enzyme inhibition assay.

Methods

Plant Material

I. aquatica was grown at research farm of Universiti Teknologi Malaysia, Pagoh, Johor, Malaysia. Organic and inorganic compound fertilizer was applied at first and second week after planting according to nutrient requirement of plant with irrigation twice a day. The plant was harvested on week four.

Preparation of Crude Extract

The stem and leaves of *I. aquatica* was oven dried at 40°C. The dried sample (500 g) was ground using a domestic blender. The sample was soaking in 1 L methanol at room temperature in the dark for 48 hours with occasional agitation. The crude extract was filtered twice through filter paper (Whatman No. 1). The filtrate was then evaporated to dryness using rotary evaporator at 40°C. The dried crude extract was stored in airtight container at 4°C.

Lipoxygenase Inhibition Assay

Ability of extracts in inhibiting lipoxygenase activity was measured using spectrophotometric assay (Malik et al., 2004). A volume of 160 µL sodium phosphate buffer (0.1 M, pH 8.0) was mixed with 10 µL of extract (dissolved in DMSO) and soybean lipoxygenase solution (20 µL). The reaction mixture incubated for 10 minutes at room temperature. Next, the reaction mixture was added with 10 µL linoleic acid as substrate. The absorbance was then recorded at 234 nm. Control reaction contains 10 µL of DMSO in place of extract solution. The percentage of lipoxygenase inhibition activity by *I. aquatica* crude extract was calculated using equation (1) below:

Inhibition activity (%): $(A-B)/(A) \times 100$.....(1)
Where, A is an absorbance value of control reaction and B is an absorbance value of sample.

Hyaluronidase Inhibition Assay

Hyaluronidase inhibition activity of extract was performed using spectrophotometric assay (Ling et al., 2003). A volume of 25 µL extract (dissolved in DMSO) was mixed with 80 U hyaluronidase, 100 µL of sodium phosphate buffer (20 mM) and incubated at 37°C for 10 minutes. Then, 100 µL of hyaluronic acid was added to the reaction mixture and further incubated for 45 minutes at 37°C. Next, 1 mL of acid albumin was added to precipitated undigested hyaluronic acid. The mixture was further incubated for 10 minutes at 24°C. After incubation, the reaction absorbance was recorded at 600 nm. Flavonoid apigenin was used as a standard compound. The absorbance of reaction mixture without enzyme was established as reference value. The percentage of hyaluronidase inhibition activity by *I. aquatica* crude extract was calculated using equation (2) below:

Inhibition activity (%): (A)/(B) × 100.....(2)
Where, A is absorbance of test sample and B is absorbance of reference reaction.

Xanthine Oxidase Inhibition Assay
Inhibition of xanthine oxidase by *I. aquatia* extract was evaluated in a 96-well plate (Fariza et al., 2012; Noro et al., 1983). Potassium phosphate buffer (130 µL, 0.05 M, pH 7.5) was mixed with 10 µL of extract and 10 µL of xanthine oxidase solution. The mixture was incubated at 25°C for 10 minutes. Xanthine solution (100 µL) was then added to initiate the reaction. The conversion of xanthine to uric acid and hydrogen peroxide was measured at 295 nm. Allopurinol was used as standard compound. All data were obtained from triplicates analysis. The percentage of xanthine inhibition activity was calculated based on equation (1).

Statistical analysis
Data were represented as mean value ± standard deviation from triplicates analysis. One-way ANOVA and post-hoc test were performed using IBM SPSS Statistics 23 software. A p value <0.05 were deemed as statistically significant.

Result and Discussion

In this study, *I. aquatica* was extracted using methanol due to higher yield in total phenolic content and antioxidant activity compared with other solvent such as hexane, chloroform and water (Prasad et al., 2005). This is the first study reported on lipoxygenase, hyaluronidase and xanthine oxidase inhibitory activity by crude extract from *I. aquatica*. Lipoxygenase is an enzyme that catalyzes the hydroperoxidation polyunsaturated fatty acids such as linoleic acid and arachidonic acid in leukotriene synthesis. In pathological condition, lipoxygenase is reported to be involved many inflammatory related diseases (Mashima & Okuyama, 2015). In this study, *I. aquatica* crude extract dose-dependently inhibited lipoxygenase activity with maximum percentage of inhibition by 87.18% at a concentration of 1000 µg/mL as shown in Table 1. Quercetin is present in the extract and its derivative are potent lipoxygenase inhibitor (Ha et al., 2010 and Chu et al., 2000). Previous study reported that *I. aquatica* contains rutin as one of the major flavonoids (Hefny Gad et al., 2018) and this compound showed inhibition against lipoxygenase with 75.63% at a concentration of 1 µg/µL (Gautam et al., 2016). Therefore, the presence of quercetin and rutin in the crude extract may contribute to lipoxygenase inhibitory activity.

Table 1. Effect of different concentrations of crude extract of *I. aquatica* extract on lipoxygenase, hyaluronidase and xanthine oxidase inhibitory activity

Sample	Concentration (µg/mL)	Inhibition activity (%)		
		Lipoxygenase	Hyaluronidase	Xanthine oxidase
Crude extract of *I. aquatica*	100	28.30±1.59^{a}	14.34±1.40^{a}	4.98±4.53^{a}
	500	62.69±2.07^{b}	62.27±6.54^{b}	36.90±4.17^{b}
	1000	87.18±7.17^{c}	95.36±8.03^{c}	78.38±9.33^{c}
Diclofenac	100	98.45±1.70^{d}	NA	NA
Apigenin	100	NA	98.70±2.25^{c}	NA
Allopurinol	100	NA	NA	99.52±0.34^{d}

*Data labeled with different superscript letters at each column are statistically significant different (p<0.05).
*NA denotes not applicable.

Hyaluronidase hydrolyzes polymeric hyaluronic acid to form hyaluronic acid oligosaccharides in the extracellular matrix of connective tissue. These oligosaccharides involve inflammation by upregulating CD44 receptors, various cytokines and inflammatory mediators (Bralley et al., 2007). Therefore, inhibition of hyaluronidase is useful in finding candidates for anti-inflammatory agent. *I. aquatica* crude extract showed inhibition activity against hyaluronidase in dose-dependent manner. Inhibition of hyaluronidase activity by *I. aquatica* crude extract at a concentration of 1000 ug/mL was 95.36%, meanwhile standard compound, apigenin inhibit hyaluronidase activity with maximum inhibition by 98.70% at a concentration of 100 µg/mL.

Xanthine oxidase is an enzyme that oxidizes hypoxanthine to xanthine and further converted to uric acid. These reactions generate byproducts including hydrogen peroxide and superoxide anions. These reactive oxygen species have been linked to pathogenesis of atherosclerosis (Nomura et al., 2014). Table 1 shows *I. aquatica* crude extract possessed good xanthine oxidase inhibition activity. These results showed dose-dependent manner between *I. aquatica* crude extract concentration and xanthine oxidase inhibition activity. However, positive control allopurinol has higher inhibition activity than the crude extracts. The high inhibition of allopurinol is due to nature of the single compound, which increase specificity and potency.

Besides quercetin derivatives, there are several main metabolites presence in the *I. aquatica* such as nomilinic acid glucoside, tricaffeoylquinic acid derivatives and fatty acid (Lawal et al., 2016). Based on literature search, it is currently unclear which compounds in *I. aquatica* crude extract responsible for inhibition of lipoxygenase, hyaluronidase and xanthine oxidase. The abundance of polyphenolics compounds presence in *I. aquatica* crude extract possibly contributed to inhibition activities. These compounds may interact with each other in term of synergistic, additive or antagonistic. Therefore, testing biological activity using individual compound may not correspond with the crude extract.

Conclusion

The maximum *I. aquatica* crude extract inhibited lipoxygenase, hyaluronidase and xanthine oxidase were 87.18%, 95.36% and 78.38%, respectively when the concentration 1000 μg/mL crude extract was used. Results from this study enhance current knowledge on bioactivity of *I. aquatica* crude extract particularly on anti-inflammatory activity.

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