Zanzibar yam (*Dioscorea sansibarensis* Pax) isolates exhibit cyclooxygenase enzyme and lipid peroxidation inhibitory activities

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**ABSTRACT**

Several phenanthrenes (1–5), phenolics (6–8) and steroidal sapogenins (9–11) were isolated for the first time from the aqueous and methanolic extracts of *Dioscorea sansibarensis* Pax yam collected from Tanzania. Chemical structures of all the isolates (1–11) were determined by using 1D and 2D nuclear magnetic resonance spectral methods. All pure isolates were evaluated for anti-inflammatory activity using *in vitro* cyclooxygenase enzyme (COX-1 and -2) inhibitory assays. Among the isolates tested, phenanthrenes 3–5 showed the highest COX-1 and -2 enzyme inhibitory activity whereas phenolics (6–8) and steroidal sapogenins (9–11) exhibited moderate inhibition when compared to non-steroidal anti-inflammatory drugs aspirin, ibuprofen and naproxen. Compounds 6–11 were evaluated for antioxidant activity using lipid peroxidation inhibitory (LPO) assay for the first time and exhibited moderate LPO inhibition.

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1. Introduction

The common name 'Yam' applies to edible tubers belonging to genus *Dioscorea* (family *Dioscoreaceae*) which contains more than 600 species (Obidiegwu et al. 2020). Yams are an important staple food item for hundreds of millions of people living in tropical
and subtropical areas. In addition to the nutritional constituents, Dioscorea species are rich in secondary metabolites namely phenols, tannins, flavonoids, alkaloids and sapo-
nins (Azeteh et al. 2019; Obidiegwu et al. 2020). They exhibit numerous health benefits such as antioxidative, antihypertensive, anticancer, anti-inflammatory, antimicrobial, inhibition of lipid peroxidation and antidiabetic activities.

The yam D. sansibarensis, commonly known as Zanzibar yam, is native to the low-
land tropics of Africa and Madagascar. In recent years, it is also reported from tropical America, Singapore, Indonesia, Taiwan and southern China (Hsu and Wang 2012). Water soluble and toxic isoquinuclidine alkaloid, dioscorine, has been reported from various yam species including Zanzibar yam. The existence of toxic dioscorine in yams is associated with bitter taste and has been demonstrated to induce dizziness, nausea and vomiting. Dioscorine has also shown to trigger fatal paralysis of the central nervous system when ingested in large quantities (Issae et al. 2017). However, during the drought periods Zanzibar yams are consumed by the indigenous people by soaking, boiling and washing it well with water. In this study, we report the isolation of 11 known compounds from the aqueous and methanolic extracts of air-dried D. sansibar-
ensis Pax tubers for the first time (Figure 1). This is also the first report of anti-inflam-
natory activity of compounds 1–3, 6–11 and antioxidant (lipid peroxidation) activity of compounds 6–11 (Dissanayake et al. 2017; 2018).
2. Results and discussion

The chemical structure of the isolated phenanthrenes (1–5), phenolic glycosides (6 and 7), phenolic acid (8) and steroidal sapogenins (9–11) from the methanolic and aqueous extracts were deduced by 1D and 2D NMR spectral experiments. These pure compounds were identified as 2,3,4,6,7-pentamethoxyphenanthrene (1) (Leong et al. 1997), 2,4,6,7-tetramethoxyphenanthrene (2) (Coxon et al. 1982), 7-hydroxy-2,4,6,7-tetramethoxyphenanthrene (3) (Leong et al. 1997), 6-hydroxy-2,4,7-trimethoxyphenanthrene (4) (Li et al. 2016), 2,7-dihydroxy-4,6-dimethoxyphenanthrene (5) (Li et al. 2016), vanillic acid 4-β-D-glucopyranoside (6) (Li et al. 2012), 3',5'-dimethoxy-4'-O-β-D-glucopyranosyl cinnamic acid (7) (Hashimoto et al. 1992), vanillic acid (8) (Li et al. 2012), diosbulbisin A (9) (Liu et al. 2009), diosbulbisin D (10) (Liu et al. 2009) and diosbulbisin C (11) (Liu et al. 2009). Both 1H and 13C NMR spectral data of compounds 1–11 were identical to their corresponding published spectral values (Figure 1). This is the first report of compounds 1, 2, 6 and 7 from the genus Dioscorea.

Cyclooxygenases (COXs) are key enzymes that regulate the conversion of arachidonic acid into prostanoids, thromboxanes and other intermediates involved in inflammation (Dissanayake et al. 2018). Therefore, inhibition of cyclooxygenases enzymes reduces the pain and inflammation in the body. Studies have shown that selective inhibition of these COX isozymes delayed onset or diminished the progression of various inflammatory diseases. In this study, we determined the anti-inflammatory activities of all the isolated compound (1–11) by measuring the in vitro inhibition of COX-1 and COX-2 enzymes (Li et al. 2016; Dissanayake et al. 2017; 2018). Commercially available nonsteroidal anti-inflammatory drugs (NSAIDs) aspirin, ibuprofen, Celebrex® and naproxen were used as positive controls in the assays. In previous work nonsteroidal anti-inflammatory drugs (NSAIDs) aspirin, ibuprofen, Celebrex® and naproxen showed IC50 values of 1050, 25, 82 and 11 μM for COX-1 enzyme. Similarly, the IC50 values for COX-2 enzymes were 80, 6.8 and 11 μM for ibuprofen, Celebrex® and naproxen, respectively, and >1050 for aspirin (Wang et al. 1999; Kato et al. 2001). All phenanthrenes (1–5) tested exhibited good COX enzymes inhibitory activities and especially compounds 3–5 showed higher anti-inflammatory activities than aspirin, ibuprofen and naproxen. The phenolics (6–7) and steroidal sapogenins (9–11) showed COX-1 enzyme inhibition with IC50 values of 53–71 μM while indicating weak COX-2 enzyme inhibition.

The lipid peroxidation inhibitory (LPO) assay was used to evaluate antioxidant potential of isolates from Zanzibar yam. The assay detects the scavenging ability or neutralizing free radicals by compounds and extracts (Li et al. 2016; Dissanayake et al. 2017, 2018). Biochemical reactions in vivo generate free radicals and other reactive intermediates. The reaction between free radicals and vital cell components such as nucleic acids, proteins and lipids results in irreversible oxidative damage and leads to numerous diseases including arthritis, cardiovascular diseases and cancer. Antioxidants have the ability to scavenge these harmful free radicals generated in vivo and mitigate such unwanted biochemical reactions. Large unilamellar vesicles or liposome model system along with ferrous sulphate as prooxidant were used in the assay as reactants. Compounds 1–5 were not evaluated in this study due to interference of fluorescence between phenanthrene and the fluorescent probe used in the model system (Li et al.
Commercial antioxidants BHT (butylated hydroxytoluene), TBHQ (t-butylhydroquinone) and BHA (butylated hydroxyanisole) used as positive controls showed IC$_{50}$ values LPO inhibitions by 5.2, 5.5 and 5.2 μM, respectively. This is the first report of LPO inhibitory activities of compounds 6–11 and showed IC$_{50}$ values by 22.7, 18.4, 20.4, 61.6, 55.1 and 60.9 μM, respectively. These isolates were also evaluated for antioxidant activity in the MTT ((3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) assay (Dissanayake et al. 2017) and none of the compounds showed significant activity. The MTT assay is based on redox reaction and hence detects compounds with antioxidant potential that are reducing agents.

3. Conclusions

Zanzibar yam is wildly grown and not commonly eaten but consumed during shortage of food under drought conditions. Because the yams are strongly resistant to drought it also suggests that their availability could be made possible even under drought conditions caused by climate change. Although there are cultivation schemes in Nigeria and Ghana, agricultural production of Zanzibar yam is not known in other parts of the tropics. Chemical and biological activity investigations of Zanzibar yam is for the first time. Chromatographic purification results showed a cumulative amount of phenanthrenes, phenolic compounds and phytosterols in 100 g of dried Zanzibar yam as 80, 264 and 690 mg, respectively. These secondary metabolites showed anti-inflammatory activity like nonsteroidal anti-inflammatory drugs aspirin, ibuprofen and naproxen, as confirmed by their inhibition of inflammation causing COX-1 and -2 enzymes. Moderate antioxidant activity of these compounds was also evident, as indicated by the inhibition of lipid peroxidation. The in vitro bioactivity of these isolates suggests that Zanzibar yam shall be considered as a functional food. Therefore, agricultural production of this yam should provide an opportunity to market it as food with health benefits.

Disclosure statement

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