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ScienceDirect

Food Science and Human Wellness 5 (2016) 17–23

Food Science and Human Wellness

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Phenolics extract of *Tetrapleura tetraptera* fruit inhibits xanthine oxidase and Fe^{2+}-induced lipid peroxidation in the kidney, liver, and lungs tissues of rats *in vitro*

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Received 2 September 2015; received in revised form 4 November 2015; accepted 6 November 2015
Available online 17 November 2015

Abstract

The phenolics composition and inhibitory activity of *Tetrapleura tetraptera* fruit extract on xanthine oxidase (XO) and Fe^{2+}-induced lipid peroxidation in the kidney, liver and lungs tissues of rats were evaluated *in vitro*. Phenolics (flavonoids and phenolic acids) were quantified using reverse-phase high performance liquid chromatrography coupled with diode array detection (HPLC-DAD). The XO and Fe^{2+}-induced lipid peroxidation inhibitory abilities of the extract were evaluated using spectrophotometric methods. The extract contained some flavonoids and phenolic acids, including catechin, epicatechin, rutin, quercetin, luteolin, apigenin; gallic, chlorogenic, caffeic and ellagic acids that are beneficial to health. The extract inhibited XO in the kidney, liver and lungs tissues in a dose-dependent manner. The half-maximal inhibitory concentrations (IC_{50}) of the extract on XO from the tissues varied significantly ($P < 0.05$), and were in the order of liver > kidney > lungs. The extract also inhibited Fe^{2+}-induced lipid peroxidation in a dose-dependent pattern, having IC_{50} in the order of liver > lungs > kidney. *T. tetraptera* fruit extract could be a promising nutraceutical for preventing and managing hyperuricaemia and the associated disease conditions, due to its ability to inhibit XO; this bioactivity is attributable to combined effect of its flavonoids and phenolic acids.

Keywords: *Tetrapleura tetraptera*; Phenolics; Xanthine oxidase; Hyperuricaemia; Lipid peroxidation

1. Introduction

Experimental evidence has shown that elevated concentration of uric acid, otherwise known as hyperuricaemia, leads to the deposition of monosodium urate monohydrate crystals in tissue, especially joints, thereby resulting in gouty arthritis or uric acid nephrolithiasis [1,2]. Gout is a chronic inflammatory arthritis characterized by elevated concentration of uric acid in body fluids, resulting from the over-activity of xanthine oxidase (XO) [3]. It is also characterized with severe and episodic painful inflammation [4]: erythema and swelling [5]. Epidemiological studies have shown that the overall burden of the disease is increasing globally [2]. It is more prevalent in men above 30 years of age and in women older than 50 years [2,6]. Moreover, it has the propensity to reduce the quality of life of these individuals [7]. In addition to gouty arthritis, hyperuricaemia is also a well-established causative factor for uric acid kidney stones and acute kidney failure [8]. Recent epidemiologic studies have also implicated chronic mild hyperuricaemia in the development of interstitial nephritis and progressive renal failure [9]. Furthermore, it is an independent

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Peer review under responsibility of Beijing Academy of Food Sciences.

http://dx.doi.org/10.1016/j.fshw.2015.11.001

risk factor for metabolic syndrome, cardiovascular disease, hypertension, obesity, obstructive sleep apnea, stroke, vascular dementia, and preeclampsia [10].

Xanthine oxidase (XO) (EC 1.1.3.22) catalyzes the oxidation of hypoxanthine to xanthine and subsequently to uric acid [11] in the purine nucleotides catabolism. Its re-oxidation involves molecular oxygen which acts as electron acceptor, and during this reaction, superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are produced [12]. The $O_2^{\bullet -}$ is transformed into H_2O_2 and O_2 either spontaneously or by the catalytic action of superoxide dismutase. Thus, the over-activity of XO leads to the deposition of uric acid in the susceptible tissues, and this triggers the inflammatory pathways with a concomitant release of reactive oxygen species. Hence, gouty arthritis and other inflammatory diseases associated with hyperuricaemia are characterized by oxidative stress. The kidney, liver and lungs are three major organs in mammals involved in metabolism and excretion, and previous studies have reported XO activity in the tissues of these organs [6,13,14]. Functionally, in these organs materials are chemically biotransformed and the metabolic wastes, such as carbon dioxide, water, salt, urea and uric acid, are removed from the body.

Clinically, anti-inflammatory agents are used to relieve the symptoms of gout, and XO inhibitors are used to block the synthesis of uric acid. These two approaches are common treatments of gout. Allopurinol, a purine analog, is the most common XO inhibitor that functions to reduce serum urate level [1]. However, its use has some attendant side effects in patients, and for this reason it is usually contraindicated in patients with kidney or heart disease. The side effects include the risk of developing hypersensitivity syndrome that is characterized by renal impairment, hepatic dysfunction, fever, rashes, leucocytosis and eosinophilia [15]. These limitations of allopurinol have necessitated research into alternative treatment strategies for gout that could be safer and effective. In this regard, medicinal plants are widely used to treat gout, as previous studies have demonstrated that several of them with high level of flavonoids and other phenolics compounds possess XO inhibitory activity [16–18]. Plant-derived polyphenolics with antioxidant potential, including flavonoids and phenolic acids, can modulate the expression of pro-inflammatory signals and ameliorate inflammatory diseases such as arthritis [19,20].

Tetrapleura tetraptera, called "aidan" in the South-western part of Nigeria, and "ihokiriho" by the Ngwa people in the South-eastern part of Nigeria, is a deciduous tree belonging to the family Mimosaceae. It is generally distributed in the lowland forest of tropical Africa. The fruits, made up of a fleshy pulp with small, brownish-black seeds, are green when tender and dark brown when fully ripe and possess high nutritional value [21]. When dry, they have a pleasant aroma, and therefore are used as spice in Central and West Africa [22]. This spicy property makes them valuable for preparing soup for nursing mothers from the first day of birth to prevent post-partum contraction [23]. Previous studies have demonstrated that different parts of the plant are used in ethnomedicine for the treatment of several ailments including diabetes mellitus, hypertension, intestinal parasites, malaria, asthma, epilepsy, schistosomiasis, wound healing and arthritis [24,25]. Recent studies have also revealed that the pod possesses antioxidant and amylase inhibitory activities [21]; the fruits and barks extracts also have antioxidant activities [22].

The aforementioned health benefits of *T. tetraptera* make it a promising functional food. Interestingly, functional foods of plant origin have continued to receive considerable research attention in the recent time due to their nutritional quality, therapeutic effects and presumed safety [26]. Hence, to further explore the health benefits of *T. tetraptera*, the present study characterized the phenolics of *T. tetraptera* fruit, and evaluated the XO inhibitory activity of its phenolic extract in the kidney, liver and lungs tissues of rats *in vitro*.

2. Materials and methods

2.1. Samples collection and preparation

Ripe fruits samples of *T. tetraptera* were harvested from the plant in Umueze area of Ekwereazu-Ngwa village in Obi-Ngwa local government area of Abia State, Nigeria, in November, 2014. The fruits were botanically identified and authenticated at the herbarium of the Department of Botany, University of Ibadan, Nigeria. Subsequently, they were sun-dried for seven days, and the seeds were manually removed. The fruits were later milled into a fine particle size (0.5 mm) powder and packed in air-tight plastic vials, and stored at −4 °C until analysis.

Methanol, formic acid, gallic acid, chlorogenic acid, caffeic acid and ellagic acid purchased from Merck (Darmstadt, Germany). Catechin, epicatechin, quercetin, rutin, apigenin and luteolin; xanthine and allopurinol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used for analysis were of analytical grade.

2.2. Preparation of polyphenolics extract

Polyphenolics extract of *T. tetraptera* fruits was prepared according to the method described by Kuo et al. [27]. A portion of *T. tetraptera* fruits powder (100 g) was extracted three successive times with 300 mL of methanol at 50 °C for 3 h, and the samples were filtered after each extraction with Whatman (No. 2) filter paper. The combined extract was partitioned with 200 mL hexane in a separatory funnel to remove the lipids and some of the pigments. The aqueous phase was extracted three times with 180 mL ethyl acetate; after which it was evaporated to dryness at 45 °C under reduced pressure in a rotary evaporator. The residue from this step was redissolved in 250 mL water, and lyophilized to obtain approximately 5 g of *T. tetraptera* fruits polyphenolics extract. The percentage yield of the extract was 5%.

2.3. Quantification of phenolic compounds using HPLC-DAD

High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped