Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

In vitro xanthine oxidase inhibitory activity of methanol extracts of *Erythrina indica* Lam. leaves and stem bark

Kandhasamy Sowndhararajan*, Jince Mary Joseph, Dharmar Rajendrakumaran

Department of Botany, School of Life Sciences, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 16 June 2012
Received in revised form 22 June 2012
Accepted 10 November 2012
Available online 28 December 2012

Keywords:
Erythrina indica
Total phenolics
Xanthine oxidase
Stem bark

ABSTRACT

Objective: To determine the total phenolic content and *in vitro* xanthine oxidase inhibitory activity of methanol extracts of leaves and stem bark of *Erythrina indica*. **Methods:** Folin–ciocalteu method was used to determine the total phenolic content. Xanthine oxidase inhibitory activity was assayed spectrophotometrically and the degree of enzyme inhibition was determined by measuring the increase in absorbance at 295nm associated with uric acid formation. **Results:** The methanol extract of stem bark of *E. indica* contains higher level of total phenolic content (412.8 mg GAE/g extract) and also exhibited higher xanthine oxidase inhibition activity (IC_{50} 52.75 μg/mL) than the leaves. **Conclusions:** It could be concluded that the stem bark of *E. indica* was highly effective in xanthine oxidase inhibition and might be used for the gout related disorders.

1. Introduction

Gout is one of the most common metabolic disorders affecting humans and its severity is reportedly increasing over the last decades. The disease is caused by marked hyperuricemia, leading to the deposition of monosodium urate crystals in joints or kidneys, resulting in gouty arthritis uric acid nephrolithiasis[1,2]. Xanthine oxidase (XO) is an important enzyme catalyzing the hydroxylation of hypoxanthine to xanthine and xanthine to uric acid which is excreted by kidneys. Excessive production and/or inadequate excretion of uric acid results in hyperuricemi[3]. XO also serves as an important biological source of oxygen–derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging. *In vitro* bioassays are used to examine test material for XO inhibition, as inhibitors of XO may be potentially useful for the treatment of gout or other XO induced diseases[4]. Several authors reported on the XO inhibitory potential of traditionally used medicinal plants[5,6].

Erythrina indica Lam. belongs to the family Leguminosae, is an important medicinal plant, distributed in the tropical and subtropical regions over the world. Also found wild in deciduous forests throughout India and in Andaman and Nicobar Islands. Bark used medicinally as febrifuge, anti–bilious and also used to treat dysentery. Bark powder traditionally used for rheumatism, itching, fever, asthama and leprosy[7]. In cameroonian folk medicine, the root bark of *E. indica* used for the treatment of trachoma, elephantiasis, and microbial infections[8]. Different kinds of phenolic compounds including isoflavones derivatives and various biologically active metabolites were isolated from the bark of this plant[8,9]. There are numerous reports on the antioxidant and free radical scavenging potential of leaves and barks of *E. indica*[10–12]. However, there is no study pertaining to the xanthine oxidase inhibitory activity of *E. indica*. Therefore, the present study was carried out to evaluate the xanthine oxidase inhibitory potential of methanol extract of *E. indica* leaves and stem bark.

*Corresponding author: Dr. Kandhasamy Sowndhararajan, Department of Botany, School of Life Sciences, Bharathiar University, Coimbatore – 641046,
Tel.: +91–422–2428301
Fax: +91–422–2422387
E–mail address: sowndhar1982@gmail.com (Sowndhararajan, K.)

2. Materials and Methods

2.1. Sample collection and extraction

The leaves and stem barks of *Erythrina indica* were collected from Virudhu Nagar, Tamil Nadu, India. The plant was authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The sample was cleaned thoroughly, shade dried at room temperature and powdered.

The powdered sample was macerated thrice with methanol at room temperature. The combined methanol extract was concentrated by low-pressure evaporation (<40℃) and then dried in lyophilizer. The crude extract was used for the analysis of total phenolic content and XO inhibition under *in vitro* assays.

2.2. Determination of total phenolic content

The total phenolic content of methanol extract of *E. indica* leaves and stem bark was determined by Folin-Ciocalteu method. The amount of total phenolics was calculated as the gallic acid equivalents[13].

2.3. Xanthine oxidase inhibition assay (XO)

The xanthine oxidase inhibitory activity of methanol extract of *E. indica* leaves and stem bark was evaluated using the method described by Havlik *et al*[6]. The assay mixture consisted of 400 μL of 120 mmol/L phosphate buffer (pH 7.5), 330 μL of 150 μmol/L xanthine (pH 7.5), 250 μL of extract stock solution diluted to corresponding concentration in H_2O and 20 μL of enzyme solution (0.5 units/mL in buffer). The reaction was initiated by the addition of enzyme and inhibition was evaluated after 3 min. The absorption increments at 295 nm indicating the formation of uric acid were measured at 25 ℃ and the initial velocity was calculated. Inhibition of xanthine oxidase was calculated as $(1-\Delta AbsB_{lank}/\Delta Abs_{Exp})\times 100$.

3. Results

In the present investigation, the total phenolic content of the extract was expressed as gallic acid equivalents. The methanol extract of *E. indica* stem bark registered higher level of total phenolic content (412.8 mg GAE/g extract) when compared with the leaves (184.3 mg GAE/g extract).

The XO inhibitory activity of *E. indica* leaves and stem bark was evaluated by the X/XO enzymatic system. The influence of the *E. indica* extracts on XO activity evaluated by decreased production of uric acid, which was measured spectrophotometrically. The XO inhibitory activity of *E. indica* is presented in Figure 1. Both the extracts showed XO inhibitory activity in dose dependent manner. The stem bark exhibited higher activity with the IC_{50} of 52.75 μg/mL in XO enzyme inhibition when compared with the leaves (IC_{50} of 84.75 μg/mL).

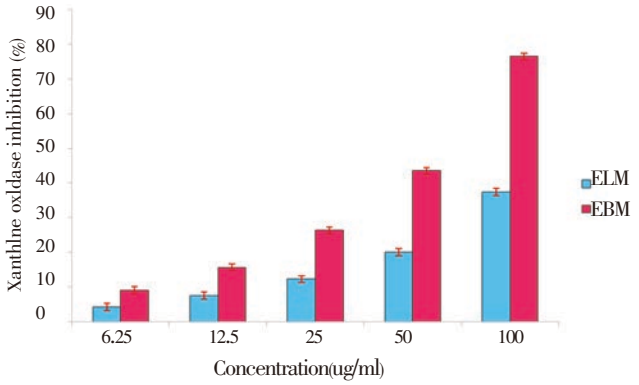

Figure 1. Xanthine oxidase inhibitory activity of methanol extracts of *Erythrina indica* leaves and stem bark. ELM – *Erythrina indica* methanol extract of leaves, EBM – *Erythrina indica* methanol extract of stem bark.

4. Discussion

The present study was aimed at discovering the XO inhibitory properties of methanol extracts of *E. indica* leaves and stem bark. Plants and their phytochemicals are potential XO inhibitors, they are used as food or food supplements for many years and found safe for human bodies[14]. In this study, methanol extract of *E. indica* stem bark possesses strong activity in XO enzyme inhibition. Related to our study, Umamaheswari *et al*[15]determined that the IC_{50} of hydromethanolic extract and its fractions of *E. stricta* were ranged between 21.2 and 100 μg/mL, and the IC_{50} of standard drug allopurinol was 6.1±0.3 μg/mL. Phenolic compounds, especially flavonoids were found as a large chemical class with XO inhibitory properties, and their role has been thoroughly evaluated[3]. Two new isoflavone derivatives together with 11 known compounds including: six isoflavones (genistein, wighteone, alpinumisoflavone, dimethylalpinumisoflavone, 8-prenyl erythrinin C, and erysenegalensein E), were isolated from the stem bark of *E. indica*[8]. Based on the previous reports, the present study clearly revealed that the XO inhibitory activity is related to total phenolic content of the extracts.

In conclusion, results obtained from this study showed that *E. indica* stem bark exhibited a good XO inhibitory activity and therefore may contain bioactive constituents useful in the treatment of XO induced diseases. Future studies are needed to isolate and identify the bioactive metabolites which effective in XO inhibitory activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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