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Journal of Ethnopharmacology 110 (2007) 92–98
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Anti-arthritic activity of a biopolymeric fraction from *Euphorbia tirucalli*

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Received 10 November 2005; received in revised form 26 April 2006; accepted 12 September 2006
Available online 26 September 2006

Abstract

The present study was undertaken to investigate the anti-arthritic activity of a biopolymeric fraction (BET) from plant *Euphorbia tirucalli* Boiss (Euphorbiaceae). The fraction showed dose dependent anti-arthritic activity and also showed *in vivo* immunomodulatory capacity being a major component in inhibiting arthritis. It caused suppression of $CD4^+$ and $CD8^+$ T cells, inhibition of intracellular Interleukin-2 (IL-2) and Interferon-gamma (IFN-γ) by flowcytometry. It inhibited vascular permeability and the migration of leucocytes at the site of the insult. The oral LD_0 in both rats and mice was more than 2000 mg/kg.

Keywords: *Euphorbia tirucalli*; Immunosuppressive; Arthritis; Flowcytometry; Intracellular; IL-2; IFN-γ

1. Introduction

Modern research in the field of anti-arthritic drugs is directed towards developing potent compounds with wide availability, non-toxicity and the ability to suppress the immune response to an antigen by oral administration [oral tolerance] (Kingsley et al., 1996). With an objective to expand the therapeutic resources within the nature, we need to understand the logic that governs the clinical or biological activity of various agents. The latex of *Euphorbia tirucalli* is used as an ointment in rheumatism, asthma, earache, cough and toothache in the traditional system of medicine (Wealth of India, 1952). This plant is an unarmed shrub that originated from Africa and is now widely grown as an ornamental plant in India (Caius, 1986). During the course of evaluation of anti-arthritic activity of native plants, the biopolymeric fraction of *Euphorbia tirucalli* was found to have significant anti-arthritic activity and was then taken up for detailed study.

Number of activities of the extracts (solvent) and pure diterpene esters isolated from *Euphorbia tirucalli* have been reported in literature (Furstemberger, 1986; Kingdom, 1979; Fursteberger, 1977; Imai, 1987; Aya, 1991). During the current decade, interest in the biopolymeric constituents of the medicinal plants has arisen because of the observations that therapeutic effect, in particular immune related activity, of a large number of plants is due to their biopolymeric constituent (Chihara et al., 1987). These constituents have emerged as an important class of natural products which act as biological response modifiers (BRMs) through modulation of immune system which in turn results in various therapeutic effects.

2. Methodology

2.1. Plant material

Euphorbia tirucalli was collected from the campus of Regional Research Laboratory (Jammu & Kashmir State, India) and authenticated by Dr. T.N. Srivastava, Taxonomist, Regional

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doi:10.1016/j.jep.2006.09.021

Research Laboratory herbarium, where voucher specimen has been deposited.

2.2. Preparation of biopolymeric fraction (BET)

The organic solvent exhausted material (0.5 kg) of the plant *Euphorbia triculli* was soaked in a 2N-aqueous sodium hydroxide and kept at 4 °C overnight. Extract was filtered and the alkaline solution centrifuged at 6000–7000 rpm at 4 °C. The above process was repeated and the aqueous alkaline solution was pooled with the first extract. The combined extract was diluted with alcohol (1:6) and kept overnight at 4 °C. The resultant precipitate was collected by centrifugation at 6000–7000 rpm and dissolved in distilled water (400 ml), acidified with equal volume of 15% aq. trichloroacetic acid and kept overnight at 4 °C. The precipitate coded as BET (12.5 g) obtained by centrifugation was suspended in warm distilled water (500 ml), and centrifuged. The aqueous solution was lyophilized. BET (10 g) was obtained as an amorphous solid.

2.3. Hydrolysis of biopolymeric fraction (BET)

BET (1.0 g) was suspended in 50 ml of aqueous 2N-TFA and then refluxed (120 °C) for 2.5 h. The reaction mixture was concentrated under reduced pressure on a film evaporator and then kept in a desiccator containing NaOH, overnight. Paper chromatography of the hydrolysed BET in comparison with reference monosaccharides was carried out in the solvent consisting of *n*-BuOH:AcOH:H_2O in the ratios of 5:1:4, respectively, and this revealed the presence of arabinose, glucose, xylose and galactose

2.4. Quantitative analysis of monosaccharides in the BET hydrolyzate by HPLC

The mobile phase used was only water, however, the HPLC column used was Rezex RPM-monosaccharide Pb++ (8%), 300 mm × 7.80 mm column, temperature 80, flow rate 0.3 ml/mm. HPLC grade water was prepared from Milli-Q water purification system. All the four monosaccharides i.e. D-glucose, D-xylose, D-galactose and D-arabinose were procured from Aldrich chemicals of purity ≤98% (HPLC).

2.5. Chromatography

Monosaccharides were separated and quantified by using Shimadzu HPLC system consisting of Pump LC-10AT*VP*, an automatic sampling unit (Autosampler), SIL-10AD*VP*, a Column oven CTO-10AS*VP*, RI detector and System controller SCL-10A*VP* version 5.40. Shimadzu.Class *VP* software version 6.10 was used for data analysis and data processing .The samples were analysed at 80 °C on a Phenomenex Rezex RPM-monosaccharide Pb++ (8%) column (300 mm × 7.80 mm) by RI detector using HPLC grade water.

Time (min)	Flow rate (ml/min)
0.1	0.3
40.0	0.3
42.0	0.5
60.0	0.5

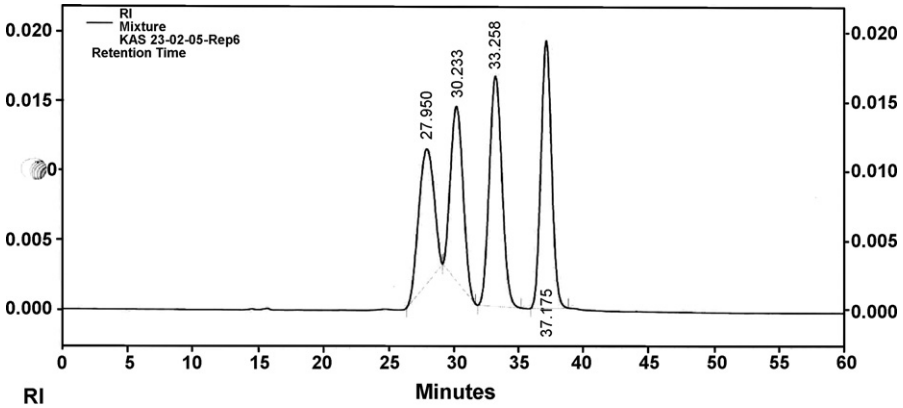
The monosaccharides were quantified by using the external standard method (Figs. 1 and 2).

2.6. Sample preparation

The accurately weighed quantity of the dried hydrolysate of BET was dissolved in known volume of HPLC grade water. The samples were filtered through millipore micro filter (0.45 μm) and then injected into the HPLC system.

2.7. Preparation of stock solutions and samples

Stock solutions of the pure reference compounds were prepared in HPLC grade water and stored in a refrigerator at 4 °C.



Monosaccharide.	R.T (min)
D-Glucose.	27.950
D-Xylose.	30.233
D-Galactose.	33.258
D-Arabinosoe.	37.175

Fig. 1. HPLC graph of D-glucose, D-xylose, D-galactose and D-arabinosoe monosaccharides.