Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 14 (2007) 534–537
Phytomedicine
www.elsevier.de/phymed

Isolation of pure compound R/J/3 from *Pluchea indica* (L.) Less. and its anti-amoebic activities against *Entamoeba histolytica*

Ria Biswas[a], P.K. Dutta[b], B. Achari[b], Durba Bandyopadhyay[a], Moumita Mishra[a], K.C. Pramanik[a], T.K. Chatterjee[a,*]

[a]*Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, West Bengal, India*
[b]*Medicinal Chemistry Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Kolkata 700 032, West Bengal, India*

Received 4 July 2006; accepted 14 August 2006

Abstract

The plant *Pluchea indica* is known for its anti-inflammatory, anti-ulcer, anti-pyretic, hypoglycemic, diuretic and anti-microbial activities besides many other pharmacological activities. We have isolated and purified seven compounds from the methanolic root extract of this plant by column chromatography. The compounds were identified by spectroscopic analyses. The anti-amoebic activities of the pure compound R/J/3 was investigated against the HM1 strain of *Entamoeba histolytica*. The compound, R/J/3 showed the most pronounced anti-proliferative activity at a dose of 50 μg/ml. It also showed a marked activity on cell lysis of trophozoites, 4 h after administration. The cell lytic activity was compared with metronidazole (5 μg/ml) as positive control.

Keywords: *Pluchea indica*; Chromatography; Anti-amoebic; *Entamoeba histolytica*; Lysis

Introduction

The use of plant extracts and phytochemicals, both with known anti-microbial properties can be of great significance in therapeutic treatments.

Amoebiasis is a ubiquitous disease, endemic to Asia, Africa and South America, is an infection by *Entamoeba histolytica*, affecting young to middle-aged adults. In most infected individuals, trophozoites exist as commensals in the large intestine, whereas in some the parasites invade the intestinal mucosa, producing mild to severe colitis (Tracy and Webster, 1996). *Pluchea indica* (L.) Less also known as Munjhu rukha or Kakronda in Bengali, belongs to the family Compositae or Asteraceae. The plant is also known to be used in rheumatoid arthritis (Chatterjee, 1996). The root extract has also been evaluated to possess anti-inflammatory (Sen and Nag Chaudhuri, 1991) and anti-ulcer (Sen et al., 1993) activities. The plant has also been reported to possess hypoglycemic (Pramanik et al., 2007) as well as diuretic (Pramanik et al., 2006) effects. So far, a number of chemical constituents have been isolated from different parts of the plant. Two new thiophene derivatives, besides two pentacyclic triterpenes of rare occurrence from roots (Chakravarty and Mukhopadhyay, 1994), have been isolated from this plant.

In the present communication, the methanolic extract of the root of the *P. indica* has been shown to yield one

*Corresponding author. Tel.: +9133 93308 43263; fax: +9133 24514782.
E-mail address: tkchatterjee81@yahoo.co.in (T.K. Chatterjee).

0944-7113/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2006.11.003

compound, namely R/J/3, on purification by column chromatography. The identity of the compound was established by spectroscopic analyses. The present study demonstrates the in vitro effectiveness of the compound R/J/3 against *E. histolytica*. The anti-amoebic activity was compared with metronidazole (5 μg/ml) as positive control. For a comprehensible understanding, the phytochemical and the anti-amoebic evaluations have been separately treated.

Isolation, purification and characterization of pure compound R/J/3 from *P. indica* (L.) Less

Isolation and purification of some compounds from methanolic root extract of *P. indica* (L.) Less. have been reported earlier (Biswas et al., 2005), (Biswas, 2006).

A portion (29 gms) of concentrated crude extract (45 gms) was partitioned between *n*-butanol and water. The crude residue of butanol fraction was shaken with ethyl acetate soluble part. The concentrated ethyl acetate extract (8.18 gms) was chromatographed over silica gel using petroleum–ethyl acetate mixtures of increasing polarity as eluants. Elution with pet–ether ethyl acetate (6:4, 4:6) yielded a solid (1.2 gms), which further yielded a yellowish solid (0.5 gms) by column chromatography over silica gel (35 gms). When eluting with pet–ether-ethyl acetate (8:2, 7.5:2.5). This solid could be resolved by column chromatography over silica gel (15 gms), by elution with pet–ether-ethyl acetate (8.5:1.5) to yield the compound R/J/3 (Fig. 1), a light yellow solid (5 gms).

Results and discussion

R/J/3

Crystallized from C_6H_6 as light yellow needles (50 mg). M.P. 106–108 °C. The sample R/J/3 in IR showed ν_{max}^{KBr} cm^{-1}: 3328 (br), 3104, 2956, 2923, 2872, 2150, 1778, 1451, 1322, 1186, 1080 (s), 1022, 946, 864, 805 (m), 688. The compound showed IR absorptions for alcoholic groups (strong bonds at 3328 and 1080 cm^{-1}). Small but significant peaks at 3104 and 2150 cm^{-1} were indicative of an unsaturated system with a triple bond. EI-MS showed signals at *m/z* (rel. abundance %): 230 (M^+, 90), 199 (100), 171 (33), 170 (32), 169 (33), 145 (22), 139 (20), 127 (50). The mass spectrum showed a strong molecular ion peak at *m/z* 230, the base peak at *m/z* 199 and another strong peak at *m/z* 169 which agreed with the presence of a CHOH-CH_2OH moiety. The In ^{1}H NMR δ^{TMS} ($CDCl_3$, 300 MHz): 1.64 (D_2O exchangeable, OH merged with solvent H_2O), 2.04 (3H, s), 3.78 (1H, dd, J = 11.4, 6.3 Hz, H_A of CH–CH_2OH), 3.82 (IH, dd, J = 11.4, 3.9 Hz, H_B of CH–CH_2OH), 4.69 (1 H, dd, J = 6.3, 3.9 Hz, CHOH–CH_2OH), 7.04, 7.18 (2H, m, thiophene-H). The ^{1}H NMR spectrum showed peaks for a methyl attached to unsaturation (δ 2.04, s), a CHX–CH_2Y unit and an aromatic system. Finally, the compound was identified as 2-(prop-1-ynyl)-5-(5,6-dihydroxyhexa-1,3-diynyl)-thiophene (Fig. 1) (Chakravarty and Mukhopadhyay, 1994).

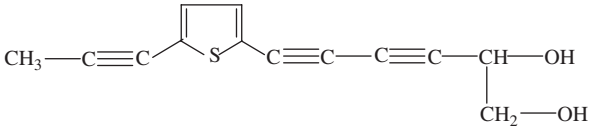

Fig. 1. R/J/3, 2-(prop-1-ynyl)-5(5,6-dihydroxyhexa-1, 3-diynyl)-thiophene.

Anti-amoebic activities of the compound R/J/3 and comparison with metronidazole (positive control)

In the present study, standardized in vitro tests, needed for screening trials, were employed to investigate the anti-proliferative action of the one pure compound R/J/3.

Materials and methods

Preparation of the *E. histolytica* culture

Forty-eight hours prior to the experiment, the culture of the HM1 strain of *E. histolytica* was transferred to the glass surface from the plastic surface. The strain was sub-cultured at a log phase of $9 \times 10^4/8 \times 10^4$ cells/ml, to eliminate any error due to cell lysis at lower concentrations. A total of 2 ml of the culture was taken in each flask and chilled at 4 °C in the refrigerator for 15–20 min for harvesting of the parasites.

Maintenance of *E. histolytica* HM1 cells

E. histolytica HM1 trophozoites were maintained at TYIS33 medium (Diamond et al., 1978).

Preparation of drug

Compound R/J/3 was dissolved in DMSO to obtain concentrations of 50 micro liter/ml and 25 micro liter/ml.

Measurement of the number of *E. histolytica* HM1 cells

A total of 8 ml of solid medium with the harvested parasites was pulled down in each tube. To each of these tubes the compound were added at concentrations of