ANTIPROTOZOAL AND ANTIBACTERIAL PROPERTIES OF
DECACHAETA INCOMPTA

FERNANDO CALZADA*, LILIAN YEPEZ-MULÍA*, AMPARO TAPIA-CONTRERAS*, ALFREDO ORTEGA*

(Received April 2009; Accepted May 2009)

This paper is dedicated to Professor Doctor Rachel Mata for her 60th birthday

ABSTRACT

We have found that a dichloromethane (DCM) extract from leaves of Decachaeta incompta exhibit antiprotozoal activity on Entamoeba histolytica and Giardia lamblia trophozoites with IC\(_{50}\) values of 132.5 μg/mL and 141.4 μg/mL, respectively. In contrast, it exhibited weak antibacterial activity on Escherichia coli, Shigella sonnei, and S. flexneri isolates. Bioassay-guided fractionation of crude extract resulted in the isolation of two known sesquiterpene lactones: incomptines A and B. Incomptine A was the most potent antiamoebic and anti giardial compound with IC\(_{50}\) values of 2.6 μg/mL for E. histolytica and 18.1 μg/mL for G. lamblia. Its potency against E. histolytica was close that of emetine (IC\(_{50}\) 1.05 μg/mL), but far less potent than metronidazole. Incomptine B showed weak activity against both protozoa but exhibited activity against all the tested bacteria with minimum inhibitory concentration (MIC) values ranging between 0.4 and 0.8 mg/mL. Its activity was superior to that chloramphenicol (MIC> 1). Incomptine A was inactive toward all tested bacteria at the concentration tested (MIC>5 mg/mL). Our work constitute the first report of antimicrobial activity of DCM extract, fractions and incomptines A and B against protozoa and bacterial isolates, which cause diarrhea and dysentery in Mexican population.

Keywords: Decachaeta incompta, Asteraceae, Sesquiterpene lactones, Antiprotozoal activity, Antibacterial activity.

RESUMEN

Nosotros encontramos que el extracto de diclorometano obtenido de las hojas de Decachaeta incompta mostró actividad antiprotozoaria contra los trofozoitos de Entamoeba histolytica y Giardia lamblia con valores de IC\(_{50}\) de 132.5 μg/mL and 141.4 μg/mL, respectivamente. En contraste, mostró débil actividad antibacteriana contra aislados de las bacterias Escherichia coli, Shigella sonnei, y S. flexneri.

*Unidad de Investigación Médica en Farmacología de Productos Naturales, CORCE, 2° Piso; †Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, UMAE Hospital de Pediatría, 2° Piso, Centro Médico Nacional Siglo XXI, IMSS, Av. Cuauhtemoc 330, Col. Doctores, CP 06725, México D. F., México

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Cd. Universitaria, Coyoacán CP 04510, México D. F., México

*Corresponding author: Tel.: (+525) 627 6900x 21367. E-mail : fercalber1@hotmail.com (PhD. Fernando Calzada Bermejo)
El fraccionamiento biodirigido del extracto condujo al aislamiento de dos lactonas sesquiterpénicas, las incomptines A y B. La incomptina A resultó ser el más potente antiamiba y antigiardia de los compuestos con valores de CI$_{50}$ de 2.6 μg/mL para *E. histolytica* y 18.1 μg/mL para *G. lamblia*. La potencia contra *E. histolytica* fue comparable a emetina (CI$_{50}$ de 1.05 μg/mL) pero menor que metronidazol. La incomptina B mostró débil actividad contra los dos protozoarios usados, sin embargo mostró actividad contra todas las cepas de bacterias empleadas con concentraciones inhibitorias mínimas (CIM) entre el rango de 0.4 y 0.8 mg/mL. Su actividad fue superior al cloranfenicol (CIM>1). La incomptina A resultó inactiva contra todas las bacterias empleadas a la concentración máxima probada (CIM> 5 mg/mL). Este trabajo constituye el primer informe de la actividad antimicrobiana del extracto de diclorometano, las fracciones y las incomptines A y B, contra protozoarios y bacterias que causan diarrea y disentería en la población mexicana.

**Palabras claves:** *Decachaeta incompta*, Asteraceae, lactonas sesquiterpénicas, actividad antiprotozoaria, actividad antibacteriana.

**INTRODUCTION**

Gastrointestinal infections are the most common cause of diarrhoea worldwide mainly in developing countries, where the rate of mortality and morbidity is very high. Diarrhoeal diseases constitute the second most common cause of death in the world with several million deaths annually. The World Health Organization has estimated that 1.5 billion episodes of diarrhoea occur every year in underdeveloped countries, resulting in 3 million deaths (Farthing, 2000; 2006; Field, 2003; Alper, 2003; Pérez et al., 2007). During past six years in Mexico, the gastrointestinal infections have been a serious health problem and were the second cause of morbidity among all age groups (SS, 2008). Patients with infectious diarrhoea present clinically one of three major clinical syndromes: acute watery diarrhoea, bloody diarrhoea (dysentery) or persistent diarrhoea (Otshudi et al., 2000; Amstrong & Cohen, 1999; Taylor et al., 1995). A number of different parasites and bacteria can cause diarrhoeal diseases; these include the protozoa *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium parvum*; and the bacteria *Escherichia coli*, *Shigella flexneri*, *Shigella sonnei*, and *Salmonella typhi* (Upcroft & Upcroft, 2001; Polombo, 2006).

Although several antimicrobial drugs are available, at present, their use is limited by a number of factors, such as low potency, emergence of resistant strains, toxicity, and side effects (Farthing, 2000; 2006; Aburjai et al., 2001; Upcroft & Upcroft, 2001; Pérez et al., 2007). Therefore, there is a distinct need for the discovery of new, safer, and more effective biologically active compounds from other sources, such as medicinal plants.

Members of the family Asteraceae (also referred to as the family Compositae) are used for diarrhoea, laryngitis and body pain. *D. thieleana*, a plant of genus *Decachaeta* is reputed to have excellent medicinal value to treat stomach aches and diarrhoea (Huang & Baker, 2001). The large number of sesquiterpene lactones and flavonoids that are typical constituents of these plants might be partly or wholly responsible for these effects (De Luengo & Mabry, 1986).

The species *Decachaeta incompta* (Asteraceae) has been used in local traditional medicine from Oaxaca, Mexico to treat diarrhoea (private communication from a local herbal healer to A. O.). In previous phyto-
chemical work, the heliangolide type sesquiterpene lactones incomptines A and B were isolated of acetone extract of the leaves from *D. incompta*. In that study, incomptine B was found to possess phytotoxic, antibacterial, antiprotozoal, and spermatogenic properties (Guerrero *et al.*, 1994). This work was undertaken to complete the pharmacological profile of this species. Herein, we report by first time the antimicrobial activity of the DCM crude extract, fractions and incomptine A. In addition at expanding the previous antimicrobial study by testing of incomptine B on bacteria and protozoa strains of clinical interest, with the aim of finding novel antimicrobial compounds of plant origin.

**MATERIAL AND METHODS**

**General procedures**

Melting points were determined using a Fisher Johns apparatus and are uncorrected. NMR spectra were recorded on a Varian Unity INOVA 500 MHz or Eclipse Jeol 300 MHz NMR spectrometers in CDCl₃ with TMS as internal standard. The chemical shifts are reported in δ units (ppm). Analytical TLC was performed on precoated Si gel (Si gel 60, Merck F₂₅₄), using a mixture of CHCl₃: EtOAc (7.5:2.5, v/v) as mobile phase. Compounds were visualized by spraying with a 10 % solution of H₂SO₄ in water and then heating at 120 °C.

**Plant materials**

The aerial parts of *Decachaeta incompta* (DC) R.M. King & H. Rob. (syn.: *Eupatorium incomptum*; Asteraceae) were collected in November 2006, in Portillo de Nejapa, State of Oaxaca, Mexico. The plant was identified by Jose Luis Villaseñor Rios, from the Herbarium MEXU of Instituto de Biologia de la UNAM where the voucher specimen (No: MEXU 1198517) was deposited.

**Extraction and isolation of heliangolides from Decachaeta incompta**

The air-dried aerial parts (27 g) were ground and extracted by percolation at room temperature with dichloromethane (DCM). After filtration, the extract was concentrated under vacuum to yield 2.12 g of green residue. The active extract (2.0 g) was subjected to column chromatography (CC) over silica gel (22.0 g, Silica gel, 70-230 mesh, Merck) using the solvent gradient system: hexane (100) and dichloromethane/MeOH (100: 0, 97: 3, 95:5, v/v). Ten fractions (50 mL each fraction) were collected and pooled on basis of their TLC profiles to obtain eight fractions (F1-F8). The antiprotozoal and antibacterial activities were located in fractions F3 and F6, respectively. Both fractions were purified by crystallization to obtain incomptine A (109 mg) and incomptine B (588 mg), respectively (Figure 1). Incomptines A and B were identified by comparison (NMR and TLC) with authentic samples. The dry material (extract and fractions) free of solvent were later redissolved in dimethyl sulfoxide (DMSO) and assessed in antiprotozoal and antibacterial assays. Incomptine A: white crystals; R: 0.60; mp 176-177 °C. Incomptine B: white crystals; R: 0.20; mp 179-180 °C.

**Figure 1.** Structural formula of incomptines A and B isolated from *Decachaeta incompta*.
Antiprotozoal assays

Entamoeba histolytica strain HM1-IMSS and Giardia lamblia isolated IMSS: 8909: 1 were used in all experiments. E. histolytica was maintained in TYI-S-33 medium supplemented with 10% bovine serum. G. lamblia trophozoites were cultured in TYI-S-33 modified medium supplemented with 10% calf serum and bovine bile. The trophozoites were axenically maintained and for the assays were employed in the log phase of growth. Both protozoa strains used in this study are cause of dysentery or diarrhoea in Mexican population (Cedillo-Rivera et al., 1991; 1992).

In vitro susceptibility assays were performed using a subculture method previously described (Calzada et al., 1998). Briefly, E. histolytica (6 x 10^3) or G. lamblia (5 x 10^3) trophozoites were incubated for 48 h at 37 °C in the presence of different concentrations (2.5-200 µg/ml) of the crude extracts in dimethyl sulfoxide. Concentration used of DMSO is non-toxic to both protozoa. Each test included metronidazole and emetine as positive controls, a control (culture medium plus trophozoites and DMSO), and a blank (culture medium). After incubation, the trophozoites were detached by chilling and samples of each tube were subcultured in fresh medium for another 48 h, without antiprotozoal drugs or extracts. The final number of parasites was determined with a haemocytometer and the 50% inhibitory concentration (IC_{50}) was calculated by probit analysis. The experiments were performed in duplicate for each protozoan and repeated at least three times. Metronidazole was used as positive control because in Mexico and other developing countries is the drug of choice currently used in the treatment of amoebic dysentery and giardiasis. In the case of emetine is an antiamoebic agent of plant origin.

Antibacterial assays

The samples were tested against six microorganisms, two Escherichia coli species, two Shigella sonnei species, and two Shigella flexneri species. The bacterial inoculum of each strain was obtained from fresh colonies grown on Muller-Hinton agar plates (MHA, Sigma). All bacterial strains used in this study were isolated from the faeces of children with acute diarrhoea or bloody diarrhoea (Torres et al., 1995). Also, all strains tested are resistant to chloramphenicol, and Shigella sonnei species are resistant to trimethoprim.

The determination of minimum inhibitory concentration (MIC) of samples was accurately determined by agar dilution technique (Alanís et al., 2005). Briefly, the extract, fractions and pure compounds for testing were dissolved in DMSO and serially diluted in melted MHA plates (100 mm x 15 mm) to obtain the final concentrations: 0.5, 1, 2, 4 mg/ml (extract and fractions) or 50 to 400 µg/ml (pure compounds). The solvent did not exceed 1 % concentration and did not affect the growth of any of the microorganisms. The cultures were diluted in Muller-Hinton broth (MHB, sigma) at a density adjusted to a 0.5 McFarland turbity standard [1.5 x 10^8 colony-forming units (CFU)/ml]. The inoculum was added to a Steer’s replicator calibrated to deliver 10^4 CFU. Then Petri dishes were inoculated and incubated at 37 °C, examined after 24 h and further incubated for 72 h. Chloramphenicol and trimethoprim were used as references standards and for comparative purpose. The lowest concentration of the sample in a plate that failed to show any visible macroscopic bacterial growth was considerer as the MIC. The MIC determination was performed in duplicate for each organism, and the experiment was repeated two times.

RESULTS AND DISCUSSION

Infectious diarrheal diseases caused by protozoa and bacteria are responsible for considerable morbidity and mortality
Antiprotozoal and antibacterial properties of *Decachaeta incompta*  


The problems of treating of gastrointestinal diseases by chemotherapy are well known. Therefore, new, safer, and more effective drugs are necessary. In this context medicinal plants have made and are continuing to make important contributions to this area of therapeutics.

In the present study, the leaves from *Decachaeta incompta*, collected in the State of Oaxaca, Mexico were extracted exhaustively with DCM. Here, we report by first time the antimicrobial activity of DCM extract, fractions and incomptine A against protozoa and bacterial isolates, which cause diarrhea and dysentery in Mexican population. In addition at expanding the previous antimicrobial study by testing of incomptine B on pathogenic bacteria and protozoa strains clinically isolated from the faeces of children with diarrhoea or dysentery (Torres et al., 1995; Cedillo et al., 1991; 1992). The result of the antiprotozoal and antibacterial activity of the DCM extract, fractions and compounds from *D. incompta* against two protozoa and six gram-negative bacteria are given in Table 1. The in vitro results were classified as follows: if the samples displayed an IC\(_{50}\) less than 20 µg/ml, the antiprotozoal activity was considered good, from 20 to 150 µg/ml the antiprotozoal activity was considered moderate, from 150 to 400 µg/ml the antiprotozoal activity was considered weak, over 400 µg/ml the samples were considered inactive (Calzada et al., 1998; 2006). In the case of antibacterial activity if the samples showed an MIC lower than 0.1 mg/ml, the activity was considered good, from 0.1 to 0.4 mg/ml the antibacterial activity was considered moderated, from 0.4 to 1 mg/ml the antibacterial activity was considered weak, over 1 mg/ml the samples were considered inactive. The DCM extract displayed moderated antiamoebic and antigiardial activity with IC\(_{50}\) values of 132.5 µg/ml for *E. histolytica* and 141.4 µg/ml in the case of *G. lambliia*. In contrast to antiprotozoal activity, the DCM extract exhibited weak antibacterial activity on *Escherichia coli*, *Shigella sonneti*, and *S. flexneri* strain. As a result of this finding, the DCM extract was fractioned by column chromatography over silica gel into eight fractions (F1-F8). As result of this process, a strongest antiprotozoal activity against both protozoa was located in F3 fraction, while the F6 fraction showed weak antibacterial activity against five bacteria tested (MIC from 0.4 to 0.9 mg/ml). After crystallization process the sesquiterpene lactones, incomptines A and B were isolated from fractions F3 and F6, respectively.

The *in vitro* antiprotozoal assay showed that incomptine A was the most potent antiamoebic and antigiardial compound with IC\(_{50}\) values of 2.6 µg/ml for *E. histolytica* and 18.1 µg/ml for *G. lamblia*. Its effect on *E. histolytica* was close to that of emetine, but far lower than that of metronidazole, the antiprotozoal drugs used as control. To our knowledge, this is the first report of antiprotozoal and antibacterial activity of incomptine A. In the case of the antibacterial assay, it showed that incomptine B was the most active compound with MIC values ranging from 0.4 to 0.8 mg/ml. Its activity against all the bacteria tested was higher than that of chloramphenicol but it did not exceed that of trimethoprim, except against *Shigella sonnei* strain. This is the first evaluation of incomptine B against *G. lamblia* and bacterial pathogen isolates. These results suggest that the heliangolide incomptine A could be considered a promising antiprotozoal drug. On the other hand, although the data are limited, the structure-effect correlation revealed that the α-methylene-γ-lactone moiety was not responsible of the antiamoebic and anti-igiardial activity. Hence, the antiprotozoal activity on *E. histolytica* and *G. lamblia* seems to be related to presence the 8-acetyl group of the heliangolide structure as is the case of incomptine A. Incomptine B which possesses a free hydroxyl at C-
8 showed weak antiprotozoal activity. In contrast, the antibacterial activity on gram-negative bacteria seems to be related to the presence of the free hydroxyl at C-8 of the sesquiterpene lactone structure. Differences in the antimicrobial effect of the isolated compounds against protozoa and gram-negative bacteria may be due to differences in permeability barriers. Further studies are needed in order to elucidate the mechanism of action of these compounds. In addition, antimicrobial properties of *D. incompta* and incomptine A suggest its potential usefulness in traditional medicine for the treatment of diarrhoea, which is caused by intestinal protozoa.

**ACKNOWLEDGEMENTS**

The authors wish to thank QFB Shan-thall Becerra for her technical assistance, Universidad del Valle de México, Campus Chapultepec.

**REFERENCES**


### Table 1. Antimicrobial activity of dichloromethane extract, active fractions and pure compounds from *D. incompta*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50} µg/mL (CI)^a</th>
<th>MIC (mg/ml)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Extract</td>
<td>132.5 (132.9-132.0)</td>
<td>141.4 (141.6-141.26)</td>
</tr>
<tr>
<td>F3</td>
<td>75.5 (75.8-75.2)</td>
<td>89.4 (89.5-89.2)</td>
</tr>
<tr>
<td>F6</td>
<td>205.2 (207.3-203.2)</td>
<td>165.7 (165.9-165.4)</td>
</tr>
<tr>
<td>Incomptine A</td>
<td>2.6 (2.7-2.4)</td>
<td>18.1 (18.4-18.3)</td>
</tr>
<tr>
<td>Incomptine B</td>
<td>137.6 (138.3-136.9)</td>
<td>256.7 (257.8-255.9)</td>
</tr>
<tr>
<td>Emetine^c</td>
<td>1.05 (1.06-1.03)</td>
<td>0.41 (0.42-0.40)</td>
</tr>
<tr>
<td>Metronidazole^c</td>
<td>0.04 (0.10-0.03)</td>
<td>0.21 (0.27-0.14)</td>
</tr>
<tr>
<td>Chloramphenicol^c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trimethoprim^c</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

^aResults are expressed as mean (n = 6), CI = 95% confidence intervals; ^bResults are expressed as mean (n = 4); ^cPositive control; A: *Entamoeba histolytica*, B: *Giardia lamblia*, C: *Escherichia coli*-1, D: *Escherichia coli*-2, E: *Shigella sonnei*-1, F: *Shigella sonnei*-2, G: *Shigella flexneri*-1, H: *Shigella flexneri*-2.
Antiprotozoal and antibacterial properties of *Decachaeta incompta* Rev. Latinoamer. Quím. 37/2 (2009) 103


