Parasitol Res (2012) 110:2351–2362
DOI 10.1007/s00436-011-2771-z

ORIGINAL PAPER

Anthelmintic activity of medicinal plants used in Côte d'Ivoire for treating parasitic diseases

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Received: 22 October 2011 / Accepted: 6 December 2011 / Published online: 27 December 2011

Abstract Natural products play an important role in the discovery and development of new pharmaceuticals. In the present study, we assessed the anthelmintic properties of medicinal plants used in Cote d'Ivoire. Ethanolic extracts from 50 medicinal plants were tested in vitro against trematodes (*Echinostoma caproni*, *Schistosoma mansoni*) and nematodes (*Ancylostoma ceylanicum*, *Heligmosomoides bakeri*, *Trichuris muris*). Active extracts were evaluated for their cytotoxicity and followed up in vivo in mice harbouring adult *S. mansoni*, *E. caproni* and *T. muris* at single oral doses of 400 or 800 mg/kg. All extracts tested were active against at least one helminths species. Ten of the 65 extracts tested (15.4%) in vitro revealed activity against all helminths tested. Of 65 extracts tested in vitro at a concentration of 2 mg/ml, all caused death of schistosomula and 34.4% and 39.1% were lethal against adult *S. mansoni* and *E. caproni* 72 h post-incubation, respectively. The highest activity against *A. ceylanicum* in vitro was observed with *Sclerocarya birrea* at 2 mg/ml, which resulted in death of adult worms and inhibition of activity of third-stage larvae (L3). Of the extracts, 41.5% completely inhibited movement of *H. bakeri* L3 at minimal lethal concentration (MLC) values of 20–200 μg/ml 48 h post-incubation, and 15.4% paralysed adult *H. bakeri* at 200 μg/ml 72 h after incubation. Of the extracts, 19% resulted in death of adult *T. muris* at MLC values of 10–100 μg/ml. In vivo, none of the extracts tested revealed activity against *E. caproni*. *Olax subscorpioidea* achieved total and female worm burden reductions of 60% and 84%, respectively in *S. mansoni*-infected mice. *Combretum mucronatum* was the most active extracts in vivo against *T. muris* with a worm burden reduction of 85.3%. In conclusion, several of the medicinal plants used in Côte d'Ivoire are active against different helminths, hence might play a role in the treatment of helminthiases. Further studies are necessary to isolate the active components from these extracts.

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Introduction

Soil-transmitted helminth (STH) infections, schistosomiasis and food-borne trematodiases are chronic, disabling parasitic diseases affecting millions of people mostly in the developing world. These neglected tropical diseases mainly occur among the rural poor (Hotez and Kamath 2009; Feasey et al. 2010). Globally, an estimated 207 million people are infected with schistosomes (Steinmann et al. 2006). A population of more than a billion is affected by soil-transmitted helminthiases (Bethony et al. 2006). Finally, 40 million people are infected with food-borne trematodes. Food-borne trematodiases are emerging in several countries due to a globalization of the food supply, increased

international travel, population growth, pollution, ecological transformations or poverty (Dorny et al. 2009; Keiser and Utzinger 2009).

The consequences of these parasitic infections are manifold. STH, for example exacerbate malnourishment and can lead to anaemia, retarded growth, mental incapacity (Bethony et al. 2006). Infections with food-borne trematodes cause inflammatory lesions and tissue damage, which can result in serious secondary complications such as cholangiocarcinoma in the case of infections with *Clonorchis sinensis* and *Opisthorchis viverrini* (Keiser and Utzinger 2009).

Only a handful of drugs are available for treatment of these diseases and most of them are very old (Keiser et al. 2006, 2010; Utzinger et al. 2011). Concerns about the development of resistance arise from an increasing use of these drugs in mass drug administration programs and experiences made in the veterinary medicine, where resistance developed shortly after different of the nematocidal drugs had been introduced (Chartier et al. 2001; Kotze et al. 2002; Almeida et al. 2010). Hence new drugs should be discovered and developed.

The use of drugs derived from plants, fungi, bacteria and marine organism has a long tradition in medicine. Plants constitute a rich source of bioactive chemicals (Newman and Cragg 2007; Mondal and Khalequzzaman 2010). By 1990, approximately 80% of drugs were either natural products or derivatives inspired by natural precursors (Li and Vederas 2009). In addition, herbal medicines are generally more accessible and affordable (Fennell et al. 2004) and are an important part of the culture and traditions of many populations. In the past years, many studies have been undertaken to assess the potential of medicinal extracts against parasitic diseases (Elango and Rahuman 2011; Ghosh et al. 2011; González-Coloma et al. 2011). Many patients from resource poor settings have strong beliefs in the use and efficacy of ethnomedicines (Chinsembu and Hedimbi 2010). In Côte d'Ivoire, medicinal plants are widely used against various diseases from parasitic and non-parasitic origin such as malaria, bacterial infection, rheumatic, gastro-intestinal parasite infections, diarrhoea, dysentery, respiratory diseases and cough (Adjanohoun and Aké Assi 1970, 1979; Bouquet and Debray 1974; Koné et al. 2002; Koné and Kamanzi Atindehou 2008; Tra Bi et al. 2005; N'Guessan et al. 2009).

The aim of the present study was to investigate the effects of selected medicinal plants from Côte d'Ivoire against a broad range of helminths, including the trematodes *Schistosoma mansoni*, *Echinostoma caproni* and the nematodes *Ancylostoma ceylanicum*, *Heligmosomoides bakeri* and *Trichuris muris*. It has been demonstrated in previous studies that different plants from West Africa investigated show a good efficacy against *Haemonchus contortus*, a sheep nematode (Diehl et al. 2004; Koné et al. 2005; Kamaraj et al. 2011), *Caenorhabditis elegans*, a free-living nematode (McGaw et al. 2000, 2007; Beloin et al. 2005; Smith et al. 2009; Waterman et al. 2010) and *Strongyloides ratti* (Olounladé et al. 2011).

The anthelmintic activity of the plants was first evaluated in vitro and promising extracts followed up in vivo.

Materials and methods

Plant material

The 50 plants studied in the present work were selected based on results of a survey carried out between 1999 and 2005 in Northern Côte d'Ivoire on the use of medicinal plants for treating human parasitic diseases and associated discomforts (stomach ache, dysentery, diarrhoea (Koné 2005; Koné et al. 2002, 2005), animal parasitic diseases (Koné and Kamanzi Atindehou 2008) and on literature reports (Diehl et al. 2004; Tra Bi et al. 2005; Togola et al. 2008; N'Guessan et al. 2009). The studied plant material was harvested in Northern (Ferkessédougou), Central (Bouaké, Katiola), Western (Guiglo) and Southern (Petit Yapo) Côte d'Ivoire in 2005, 2008 and 2010. A detailed summary of the investigated plant material, including family, indications and which parts of the plants are used is presented in Table 1. All samples collected were identified at the herbarium of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS) where voucher specimens are maintained.

Preparation of plant extracts

The air-dried plant material was grounded and extracted under mechanical stirring in a tenfold excess of ethanol 90% at room temperature during 14 h. The filtrates were evaporated on a rotary evaporator (40°C), frozen and lyophilized to yield the crude extracts. Sixty-five ethanolic crude extracts were then prepared for anthelmintic screening. The lyophilized extracts were solubilised in dimethylsulfoxide (DMSO) to prepare stock solutions (200 mg/ml) for in vitro studies. For in vivo assays, extracts were suspended in a solution composed of Tween 80 (70%) and ethanol (30%) and diluted with distilled water to obtain final concentrations of 7% Tween 80 and 3% ethanol.

Animals and parasites

The life cycles of the studied parasites are maintained at the Swiss Tropical and Public Health Institute (Swiss TPH) following animal welfare regulations. In vitro and in vivo studies were carried out in accordance with these guidelines. Female mice (NMRI strain, n=90, weight ~20–22 g) and

Table 1 Traditional uses of medicinal plants from Côte d'Ivoire selected for anthelmintic screening

Plant species (Family)	Indications and plant parts used
Acacia nilotica (L.) Willd. ex Delile (Mimosaceae)	Veterinary medicine: intestinal worms, anaemia
Afzelia africana Sm. (Caesalpiniaceae)	Malaria, stomach-ache (l)
Allophyllus africanus P. Beauv. (Sapindaceae)	Malnutrition, diarrhoea (l)
Ampelocissus africana (Lour.) Merr. (Vitaceae)	Constipation, stomach-ache (r)
Annona senegalensis Pers. (Annonaceae)	Diarrhoea, dysentery, abdominal pain, malaria (r)
Anogeissus leiocarpus (DC.) Guill. & Perr. (Combretaceae)	Malaria, diarrhoea, dysentery, anemia (l, sb)
Anthostema senegalense A. Juss. (Euphorbiaceae)	Intestinal worms (sb)
Antidesma venosum E. Mey. ex Tul. (Euphorbiaceae)	Intestinal worms (sb)
Carissa edulis (Forssk.) Vahl. (Fabaceae)	Diarrhoea in infants and children (r)
Combretum mucronatum Schumach. & Thonn. (Combretaceae)	Malaria, wound healing (l)
Combretum platypterum (Welw.,) Hutch. & Dalziel (Combretaceae)	Malaria (l)
Craterispermum caudatum Hutch. (Rubiaceae)	Cough, abdominal pain (l)
Crossopteryx febrifuga (Afzel. ex G. Don) Benth. (Rubiaceae)	Diarrhoea, malaria, abdominal pain (r, l)
Diospyros mespiliformis Hochst. ex A. DC. (Ebenaceae)	Diarrhoea, vomiting (sb)
Eriosema griseum Baker (Fabaceae)	Infant cure (l)
Erythrina senegalensis DC. (Fabaceae)	Diarrhoea, dysentery, abdominal pain, malaria, and infections of urinary tract, intestinal worms, cough, tuberculosis (r, sb)
Flabellaria paniculata Cav. (Malpighiaceae)	Malaria, wound healing (l)
Flueggea virosa (Roxb. ex Willd.) Voigt (Euphorbiaceae)	Malaria, vomiting (l)
Hybanthus enneaspermus (Linn.) F.V. Muell. (Violaceae)	Malaria, aphrodisiac, cough (w)
Khaya senegalensis (Desr.) A. Juss. (Meliaceae)	Malaria, cough, intestinal worms, diarrhoea (sb)
Lannea barteri (Oliv.) Engl. (Anacardiaceae)	Diarrhoea, sterility (l)
Lophira lanceolata Van Tiegh. ex Keay (Ochnaceae)	Anaemia, diarrhoea with blood, cough, abdominal pain, anaemia (dl, sb)
Milbraedia paniculata Pax (Euphorbiaceae)	Cough (l)
Mimusops kummel Bruce ex A. DC. (Sapotaceae)	Intestinal worms (sb)
Monotes kerstingii Gilg (Dipterocarpaceae)	Intestinal worms, diarrhoea (sb, l)
Morinda longiflora G. Don (Rubiaceae)	Malaria, abdominal pain (r)
Miytragyna ciliata Aubrév. & Pellegr. (Rubiaceae)	Malaria (l)
Miytragyna inermis (Willd.) O. Kuntze. (Rubiaceae)	Intestinal worms, malaria, diarrhoea (sb, l)
Napoleona vogelii auct., p.p. Hook. & Planch. (Lecythidaceae)	Diarrhoea (l)
Olax subscorpioidea Oliv. (Olacaceae)	Intestinal worms, malaria (r)
Parinari curatellifolia Planch. ex Benth. (Chrysobalanaceae)	Dysentery, diarrhoea, wound healing (sb)
Parinari excelsa Sabine (Chrysobalanaceae)	Constipation, abdominal pain (sb)
Pavetta crassipes K. Schum. (Rubiaceae)	Malaria, anorexia (l)
Pericopsis laxiflora (Benth. ex Baker) Meeuwen (Fabaceae)	Intestinal worms, dysentery (sb)
Piliostigma thonningii (Schumach. & Thonn.) Milne-Redh. (Caesalpiniaceae)	Cough, wound healing (sb)
Pseudarthria hookeri Wight & Arn. (Fabaceae)	Cough (r)
Pseudocedrela kotsstchyi (Scweinf.) Harms (Meliaceae)	Intestinal worms, abdominal pain (sb)
Rauvolfia vomitoria Afzel. (Apocynaceae)	Malaria, fever (sb)
Sacoglottis gabonensis (Baill.) Urban. (Humuriaceae)	Abdominal pain (sb)
Sarcocephalus latifoliusa (Smith) Bruce (Rubiaceae)	Intestinal worms, abdominal pain, fever, malaria (r)
Sclerocarya birrea (A. Rich.) Hochst. (Anacardiaceae)	Abdominal pain, cough (sb)
Securidaca longepedunculata Fres. (Polygalaceae)	Intestinal worms, anorexia, urinary infections (r, l)
Starchytarpheta cayennensis (Rich.) Vahl (Verbenaceae)	Diarrhoea, dysentery (w)
Terminalia avicennioides Guill. & Perr. (Combretaceae)	Intestinal worms, diarrhoea (sb)
Thallia geniculata L. (Maranthaceae)	Anaemia (r)
Trema orientalis (L.) Blume (Ulmaceae)	Intestinal worms, diarrhoea (r, l)
Vitellaria paradoxaum C. F. Gaertn. (Sapotaceae)	Dysentery, diarrhoea (r)
Ximenia americana L. (Olacaeae)	Diarrhoea, cough, wound healing (r)
Xylopia aethiopica (Dunal) A. Rich. (Annonaceae)	Intestinal worms, diarrhoea (sb, f)
Ziziphus mauritiana Lam. (Rhamnaceae)	Intestinal worms, diarrhoea (sb)

l leaves, *dl* dried leaves, *sb* stem bark, *r* roots, *w* whole plants, *f* fruits

male Golden Syrian hamsters (n=2, weight ~100 g) were purchased from Harlan (Horst, The Netherlands), maintained under environmentally controlled conditions and acclimatized for 1 week before infection.

In vitro assays

All in vitro tests were carried out in duplicates. The experiment was repeated three times for extracts that showed an inhibitory effect.

In vitro assays against S. mansoni schistosomula *S. mansoni* schistosomula were prepared as described in previous publications (Manneck et al. 2011). Briefly, cercariae of *S. mansoni* were harvested from infected intermediate host snails (*Biomphalaria glabrata*) after exposure to light for 4 h and subsequently transformed into schistosomula (newly transformed schistosomula, NTS). NTS were kept in Basch medium (TSS 199), supplemented with 5% heat-inactivated foetal calf serum (iFCS) and 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Carlsbad, USA) at 37°C in an atmosphere of 5% CO_2 for a minimum of 3–12 h before use. NTS-suspension (100 μl) containing 500–1,000 NTS was added to each well of 48-well plates (Costar). Stock solutions of the tested extracts were diluted in TSS 199 in order to obtain final concentrations of 2 mg/ml and 200 μg/ml. The plates were incubated at 37°C in 5% CO_2. Morphological changes, paralysis or death of NTS were monitored after 24, 48 and 72 h. Extracts, which showed activity at 200 μg/ml were further studied and serial dilutions were prepared from 160 to 5 μg/ml. The minimal lethal concentration (MLC), which is the minimum concentration needed to kill all schistosomula was determined after 24, 48 and 72 h. Schistosomula exposed to the highest concentration of solvent (1% DMSO) and worms incubated with praziquantel served as controls.

In vitro assays against adult S. mansoni Only the most active extracts (MLC=5–40 μg/ml) against NTS were selected for screening against adult *S. mansoni*. Worms were collected from infected NMRI mice from the hepatic portal system and the mesenteric veins as described previously (Manneck et al. 2010). Cleaned worms (n=4; 2 males and 2 females) were placed in each well of 24-well plates (Costar), in presence of extracts at 2 mg/ml or 200 μg/ml in RPMI 1640 culture medium supplemented with 5% iFCS and 1% pencillin–streptomycin. Schistosomes incubated with the highest concentration of drug solvent served as controls. Worms were kept in an incubator at 37°C, 5% CO_2 for up to 72 h. The effect of the extracts was assessed under a dissecting microscope with an emphasis on changes in worm motility (activity or paralysis) and death of worms. Death was defined as no movement observed for at least 2 min of examination. Active extracts were further diluted (160–5 μg/ml) and tested as described above.

In vitro assays against adult E. caproni NRMI mice were orally infected with 25 metacercariae of *E. caproni* freshly collected from infected *B. glabrata*. Two weeks post-infection, mice were euthanized with CO_2 and adult worms were removed from the intestine. Two worms were placed in each well of 24-well plates (Costar) in RPMI medium supplemented with 1% penicillin–streptomycin and 20% α-D-glucose. Adult *E. caproni* were incubated in the presence of 2 mg/ml or 200 μg/ml of the extracts in 5% CO_2 atmosphere at 37°C for 72 h. Worms exposed to medium supplemented with the highest concentration of DMSO (1%) served as control. Worms were monitored daily using a dissecting microscope and changes in worm motility and morphology and death recorded. Extracts active at 200 μg/ml were diluted tenfold and re-tested.

In vitro assays against larvae of A. ceylanicum Third-stage larvae (L3) were cultured from the faeces of *A. ceylanicum*-infected Syrian Golden hamsters 20 days post-infection as described recently (Tritten et al. 2012). Thirty larvae were incubated in the presence of the individual extracts (2 mg/ml or 200 μg/ml) and 100 μl of Hank's Basal Salt Solution (HBSS) supplemented with 10% of amphotericin B and 1% of penicillin–streptomycin in 96-well plates (Costar). Control wells contained 1% DMSO only. The plates were stored at ambient temperature for 72 h. L3 were stimulated by adding 100 μl of warm water (~80°C) to the wells. The number of moving larvae was counted and the percentage of survival calculated.

In vitro assays against adult A. ceylanicum Syrian Golden hamsters were orally infected with 150 *A. ceylanicum* L3 (Tritten et al. 2012). Twenty days post infection, hamsters were euthanized with CO_2 and worms collected from digestive tract were placed in HBSS supplemented with 10% amphotericin B, 1% penicillin–streptomycin and 10% FCS. Three rinsed worms each were incubated in the presence of 2 mg/ml or 200 μg/ml of extracts in 48 well plates in 1,000 μl supplemented HBSS for 72 h at 37°C, 5% CO_2. After incubation, worms were stimulated with 500 μl of warm water (~80°C) and death recorded.

In vitro assays against larvae of H. bakeri L3 were cultured from faeces of infected NRMI mice as described in a recent publication (Nwosu et al. 2011). L3 (30 per well) were placed in 96 well plates (Costar) containing 100 μl HBSS supplemented with 10% of amphotericin B and 1% of penicillin–streptomycin and three dilutions (2 mg/ml, 200 and 20 μg/ml) of the extracts. Larvae exposed to 1% DMSO served as controls. The larvae were incubated for 72 h at room temperature.

After incubation, they were stimulated with 100 μl of hot water (~80°C) and the percentage of inhibitory motility (IM) determined. Active extracts (IM value≥80%) at 20 μg/ml were further diluted to 2 μg/ml and the activity assessed.

In vitro assays against adult H. bakeri Only extracts showing IM values of at least 80% at 20 μg/ml against *H. bakeri* L3 were tested against the adult worms. For this test, NRMI mice were orally infected with 80 *H. bakeri* L3. One week post-infection, mice were euthanized with CO_2 and adult worms collected from the intestines. Worms were rinsed with RPMI medium and incubated in 48 well plates (Costar) in RPMI medium supplemented with 5% penicillin–streptomycin and 0.2% amphotericin. Adult *H. bakeri* were incubated in the presence of extracts (200 or 20 μg/ml) at 37°C in 5% CO_2 atmosphere for 72 h. Changes in the motility and morphology of adult *H. bakeri* were monitored under a dissecting microscope.

In vitro assays against adults of T. muris Adult *T. muris* were collected from the intestines of infected NRMI mice. Worms were rinsed in RPMI and four worms were placed in individual wells of 24 well plates (Costar), containing RPMI medium supplemented with 5% amphotericin and 1% penicillin–streptomycin and the test extracts (2 mg/ml and 200 μg/ml). Plates were incubated in 5% CO_2 atmosphere at 37°C. The effect of the plant extracts on the motility and morphology was documented using a dissecting microscope after 24, 48 and 72 h following incubation. Worms were classified as dead when no movement was observed for at least 2 min of examination. Extracts exhibiting activity at 200 μg/ml were serially diluted from 160 to 5 μg/ml and tested for activity against adult *T. muris*.

Cytotoxicity test on skeletal myoblast cells using Alamar Blue assay

Cytotoxicity tests on L-6 rat skeletal myoblast cells were carried out for all active (MLC≤80 μg/ml) extracts. L-6 rat skeletal myoblast cells were seeded in 96-well microtiter plates at a density of 105 ml^{-1} in MEM supplemented with 10% heat-inactivated foetal bovine serum. Crude extracts at concentrations ranging from 90 to 0.001 μg/ml were added. The plates were incubated for 72 h at 37°C in 5% CO_2. After 70 h, 10 μl of Alamar Blue® was added to each well. After a further incubation of 2 h, the plates were read using a fluorescence reader and the IC_{50} values calculated. Podophyllotoxin was included as positive control.

In vivo assays

In vivo activity against E. caproni The eight most active extracts from the in vitro screening were selected for in vivo assays against *E. caproni*. NRMI mice were orally infected with 25 metacercariae of *E. caproni* and 2 weeks post-infection *E. caproni*-infected mice (four mice per extract) were treated with single oral doses of 800 mg/kg of the test extracts. In the first step, a high dose was used in order not to miss any activities. For comparison, 100% worm burden reductions were obtained with a single oral 50 mg/kg dose of praziquantel (Keiser et al. 2006). Four mice remained untreated and served as controls. One week post-treatment, mice were euthanized with CO_2 and dissected. Worms were removed from the intestine and counted using a binocular microscope.

In vivo activity against S. mansoni Mice harbouring chronic (49 days post-infection) *S. mansoni* infections were treated at single oral doses of 400 mg/kg with the 11 extracts, which showed the highest activities in vitro. This dose was selected since praziquantel achieved a worm burden reduction of 93% following a dose of 400 mg/kg (Keiser et al. 2011). Groups of three to four mice were used per extract. One group of untreated mice served as controls (n=8). Four weeks post-infection, mice were killed by cervical dislocation and the mesentery and the liver were collected as described previously by Manneck et al. (2011). Schistosomes were removed from the mesenteric veins, sexed and counted using a binocular microscope. The liver was flattened and examined for the presence of worms. Worm count was compared to untreated controls.

In vivo activity against T. muris NRMI mice were orally infected with 400 eggs of *T. muris*. Fourty five days post infection, four mice were treated with a single dose of 400 mg/kg of each of the five extracts, which had shown the highest activities in vitro. One week post-treatment, stools were harvested and analysed under binocular microscope for counting expelled worms. Mice were euthanized and dissected, and the intestine was examined for the presence of worms. Worm expulsion rate and worm burden reduction were calculated.

Statistical analyses

Statsdirect statistical software (version 2.4.5, Statsdirect Ltd, Cheshire, UK) was used to compare the medians of the worm counts with a Kruskal–Wallis test. Differences in medians were considered to be significant at a significance level of 0.05.

Results

All the extracts screened in this study were active against at least one of the helminth species tested, causing reduced viability, paralysis or death of the worms. Overall, ten

(15.4%) of the 65 extracts tested in vitro showed activity against all (n=5) helminths tested, hence displayed both trematocidal and nematocidal properties. The extracts with the broadest spectrum of activity were prepared from the roots and stem bark of *Anogeissus leiocarpus*, roots of *Crossopteryx febrifuga*, leaves of *Eriosema griseum*, leaves of C*ombretum mucronatum*, roots of *Monotes kerstingii*, stem bark of *Xylopia aethiopica*, roots of *Securidaca longepedunculata*, roots of *Olax subscorpioidea*, roots of *Sacoglottis gabonensis* and roots of *Erythrina senegalensis*. Of the remaining extracts, 6 (9.2%) extracts showed activity against 4 helminths, 17 (26.2%) revealed activity against 3 worms, and 19 (29.2%) and 13 (20%) were active against 2 helminths and 1 worm, respectively.

Activity against *S. mansoni* NTS and adults in vitro

The screening of plant extracts against schistosomes showed that NTS were more affected by these products than the adult worms of *S. mansoni*. Of the 65 extracts tested at 2 mg/ml, 53 (84.4%) were active against NTS. Schistosomula had died in the presence of these extracts after 24 h. This percentage increased to 98.4% and 100% 48 and 72 h post-incubation, respectively. When tested at 200 μg/ml, 28 (42.2%) extracts caused death of NTS after 24 h, 45 (70.3%) after 48 h and 54 (84.4%) after 72 h of incubation. Following further dilutions, seven extracts still showed a high activity against NTS, namely the stem bark of *S. gabonensis* (MLC=5 μg/ml), roots and stem bark of *A. leiocarpus*, leaves of *Craterispermum caudatum*, roots of *E. senegalensis*, leaves of *Lannea barteri* and stem bark of *X. aethiopica* (all MLC=10 μg/ml; Table 2). Extracts of the leaves of *C. mucronatum*, leaves of *E. griseum*, leaves of *Flabellaria paniculata*, roots of *M. kerstingii*, and stem bark of *Pseudocedrela kotsstchyi* resulted in death of NTS at 20 μg/ml.

Of the 30 extracts tested against adult schistosomes (characterized by an MLC of 5–40 μg/ml against NTS) at a concentration of 2 mg/ml, 17 (26.6%), 21 (31.8%) and 22 (34.4%) extracts caused death of adult schistosomes 24, 48 and 72 h post-incubation, respectively. Fifteen extracts and one product displayed an activity at tenfold and 100-fold lower concentrations (200 and 20 μg/ml), respectively 48 h post-incubation. The most active extracts were the roots of *C. febrifuga* with a MLC of 10 μg/ml, the leaves of *E. griseum* (MLC=40 μg/ml), the stem bark of *S. gabonensis* and the roots of *S. longepedunculata* with MLCs of 80 μg/ml (Table 2).

Activity against *E. caproni* in vitro

Fourteen (21.9%), 20 (31.3%) and 25 (39.1%) of the extracts tested caused death of *E. caproni* at a concentration of 2 mg/ml 24, 48 and 72 h post-incubation, respectively. At 200 μg/ml worms were killed by 15 (23.1%) extracts 72 h post-incubation. The highest echinostomicidal activity (MLC=20 μg/ml) was observed with extracts prepared from the leaves of *Milbraedia paniculata*, roots of *S. longepedunculata* and the stem bark of *X. aethiopica* (Table 2). These extracts caused death of the worms between 48 and 72 h and a change of morphology (colour, granulation) was observed following incubation with *S. longepedunculata* and *X. aethiopica*.

Activity against *A. ceylanicum* in vitro

With the exception of nine extracts (roots of *Ampelocissus grantii*, stem bark of *Khaya senegalensis*, stem bark of *P. kostchyi*, stem bark of *S. gabonensis*, stem bark and roots of *Sclerocarya birrea*, leaves of *E. griseum*, stem bark of *Terminalia avicennioides* and stem bark of *Ziziphus mauritiana*), which exhibited moderate activity (percentage of worms surviving ranging between 10% and 40%) 48 h post-incubation, none of the studied extracts showed an activity against *A. ceylanicum* L3 in vitro at a concentration of 2 mg/ml. At 200 μg/ml, only the root extract of *S. birrea* showed activity (survival of 36.6%) at the 48 h examination point.

Only the root extract of *S. birrea* killed adult *A. ceylanicum* 72 h after incubation, when exposed to a high concentration of 2 mg/ml (Fig. 1). At the same concentration, six extracts from *C. mucronatum* (leaves), *S. longepedunculata* (roots), *M. kerstingii* (leaves), *Z. mauritiana* (stem bark), *L. barteri* (roots) and *O. subscorpioidea* (roots) reduced the viability of adult worms and paralysed the worms (72 h post incubation), however did not result in killing of worms.

Activity against *H. bakeri* in vitro

Fourteen (23.1%) and 12 (18.5%) of the products (derived from *A. leiocarpus*, *C. febrifuga*, *E. griseum*, *Flueggea virosa*, *L. barteri*, *Lophira lanceolata*, *M. kerstingii*, *Napoleona vogelii*, *Pericopsis laxiflora*, *S. gabonensis* and *S. longepedunculata*) reduced motility of *H. bakeri* L3 by at least 80% at concentrations of 200 and 20 μg/ml 48 h post-incubation, respectively (Table 2). When extracts (IM value≥80%) were tested against adult *H. bakeri*, only ten (15.4%) extracts exhibited activity. These extracts were prepared from *M. kerstingii* (leaves), *Annona senegalensis* (roots), *T. avicennioides* (stem bark), *S. longepedunculata* (leaves), *M. paniculata* (leaves), *Mimusops kummel* (roots and stem bark), *Miytragyna ciliata* (leaves), *S. gabonensis* (roots) and of *C. mucronatum (*leaves). All ten extracts caused death of worms at 2 mg/ml 48 h post incubation. Exposure to 200 μg/ml of these ten extracts resulted in reduced movements and paralysis of worms 72 h post-incubation. The extracts from the roots of *E. senegalensis* and the stem bark of *X. aethiopica* caused twisting and death of the worms at 200 μg/ml after 72 h of incubation.

Table 2 Minimum lethal concentrations (microgrammes per millilitre) of studied medicinal plants against *E. caproni*, *S. mansoni*, *T. muris* and *H. bakeri* in vitro

Plant species	Plant part tested	Helminths tested				
		E. caproni	*S. mansoni*		*T. muris*	*H. bakeri*
		Adults	NTS	Adults	Adults	L3 larvae
Anogeissus leiocarpus	Roots	200	10	250	10	20
	Stem bark	>2,000	10	160	10	>2,000
Anthostema senegalenseis	Stem bark	>2,000	40	2,000	2,000	200
Combretum mucronatum	Leaves	>2,000	20	200	10	2,000
Craterispermum caudatum	Leaves	2,000	10	200	>2,000	2,000
Crossopteryx febrifuga	Roots	160	40	10	20	20
Eriosema griseum	Leaves	2,000	20	40	2,000	20
Erythrina senegalensis	Roots	80	10	160	2,000	200
Flabellaria paniculata	Leaves	>2,000	20	200	2,000	200
Khaya senegalensis	Stem bark	>2,000	40	1,000	2,000	>2,000
Lannea barteri	Roots	2,000	40	2,000	2,000	20
	Leaves	2,000	10	200	2,000	200
Milbraedia paniculata	Leaves	20	40	200	2,000	200
Mimusops kummel	Roots	160	80	nd	2,000	2,000
	Stem bark	2,000	40	200	2,000	20
Monotes kerstingii	Stem bark	2,000	40	160	2,000	200
	Leaves	>2,000	2,000	nd	2,000	20
	Roots	80	20	200	40	2,000
Morinda longiflora	Roots	200	200	nd	>2,000	2,000
Napoleona vogelii	Leaves	2,000	80	nd	2,000	20
Olax subscorpioidea	Roots	80	40	160	100	2,000
Parinari curatellifolia	Stem bark	>2,000	80	nd	2,000	>2,000
Parinari excelsa	Stem bark	>2,000	160	nd	100	2,000
	Leaves	>2,000	200	nd	>2,000	20
Piliostigma thonningii	Stem bark	2,000	40	2,000	>2,000	>2,000
Pseudocedrela kotsstchyi	Stem bark	>2,000	20	500	20	>2,000
Sacoglottis gabonensis	Stem bark	200	5	80	2,000	20
Sarcocephalus latifoliaus	Roots	2,000	160	nd	>2,000	2,000
Sclerocarya birrea	Stem bark	>2,000	40	2,000	100	>2,000
	Roots	>2,000	200	nd	100	2,000
Securidaca longepedunculata	Roots	20	40	80	20	20
	Leaves	200	40	250	>2,000	200
Starchytarpheta cayennensis	Whole plant	160	40	160	2,000	200
Terminalia avicennioides	Stem bark	200	40	500	20	2,000
	Leaves	>2,000	40	500	2,000	2,000
Xylopia aethiopica	Stem bark	20	10	160	2,000	200

nd non determined (MLC against NTS>40 μg/ml)

Activity against *T. muris*

Fourteen (21.5%), 37 (56.9%) and 45 (69.2%) of extracts caused death of adult *T. muris* at 2 mg/ml 24, 48 and 72 h post-incubation, respectively. Eight extracts displayed a high activity with MLC values ranging between 5 and 20 μg/ml 72 h post-incubation. These extracts were prepared from the roots and stem bark of *A. leiocarpus*, the leaves of *C. mucronatum*, the roots of *C. febrifuga*, the stem bark of *P. kotsstchyi*, the roots of *S. longepedunculata*, the stem bark of *T. avicennioides* and the roots of *M. kerstingii* (Table 2).

Cytotoxicity of the tested extracts

The evaluation of the cytotoxicity of 22 tested extracts on L6 rat skeletal myoblast cells showed either no toxicity (IC_{50}>90 μg/ml) or only a very low toxicity of 4 extracts (roots of *A. leiocarpus*, leaves of *C. mucronatum*, roots of

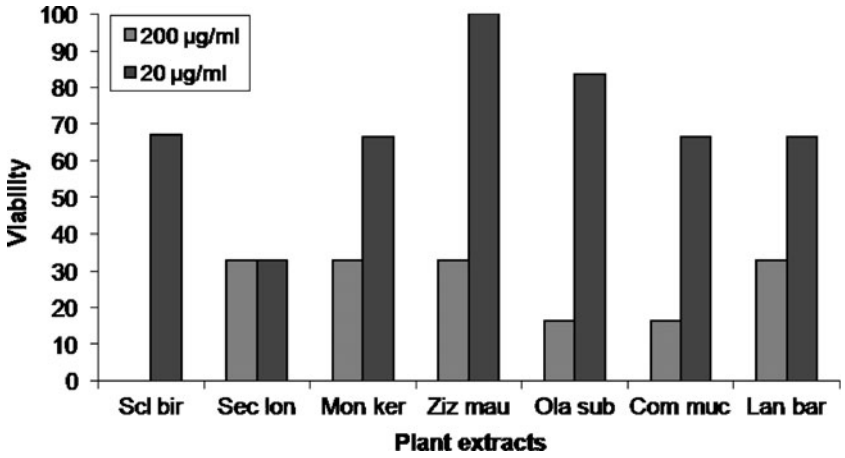

Fig. 1 In vitro effect of plant extracts on viability of adult *A. ceylanicum*. *Scl bir*, *Sclerocarya birrea*; *Sec lon*, *Securidaca longepedunculata* *Mon ker*, *Monotes kerstingii*; *Ziz mau*, *Ziziphus mauritiana*; *Ola sub*, *Olax subscorpioidea*; *Com muc*, *Combretum mucronatum*; *Lan bar*, *Lannea barteri*

O. subscorpiodiea and stem bark of *S. gabonensis*) (IC_{50} 31.8–43.3 µg/ml) when compared to the positive control (Podophyllotoxin IC_{50} 0.004 µg/ml).

In vivo anthelmintic efficacy of plants

In vivo assays were carried out with extracts, which displayed the highest activity against adult *S. mansoni* (n=11; MLC of 20–160 µg/ml), *E. caproni* (n=8; MLC=20–200 µg/ml) and *T. muris* (n=5; MLC of 10–20 µg/ml) in vitro. We did not carry out in vivo studies in *H. bakeri*-infected mice because none of the extracts revealed promising activity against adult *H. bakeri* in vitro. In addition, in vivo studies in *A. ceylanicum*-infected hamsters were not done, as only the root extract of *S. birrea* was active at a high concentration of 2 mg/ml against adult worms.

Activity against *E. caproni*

None of the tested extracts showed activity against *E. caproni* in vivo. Worm burden reductions achieved with a single oral dose of 800 mg/kg ranged between 0% and 21% for these extracts (P>0.05; Table 3).

Activity against *T. muris*

At a single oral dose of 400 mg/kg, one of the five extracts tested in vivo against *T. muris*, prepared from the leaves of *C. mucronatum* showed a significant effect (P=0.038) with a worm burden reduction of 85.3%. Low to moderate worm burden reductions (3.1–40.7 %) were observed with the remaining four extracts (Table 3).

Activity against *S. mansoni*

Low total worm burden (0–16.1%) and female worm burden (0–11.1%) reductions were documented for the extracts of *A. leiocarpus* (roots), *E. senegalensis* (roots), *M. kerstingii* (roots), *S. longepedunculata* (roots), *Starchytarpheta cayennensis* (whole plant), *S. gabonensis* (stem bark) and *X. aethiopica* (stem bark). Moderate worm burden reductions (total worm burden reduction of 49.5% and female worm burden reduction of 48.9%) were achieved with the leaves of *E. griseum*. With the stem bark of *A. leiocarpus* and roots of *C. febrifuga*, moderate total worm burden (46.3–57.0%) and high female worm burden (71.1%) were observed. The highest activity in *S. mansoni*-infected mice (total worm burden reduction of 60.2% and female worm burden reduction of 84.5% (which however was not significant since only a small number of mice were used) was observed with the roots of *O. subscorpiodea* (Table 4).

Discussion

In the present study, as many as 50 medicinal plants used in Côte d'Ivoire and elsewhere in West Africa were evaluated against a wide range of helminths, namely the trematodes *E. caproni* and S. *mansoni* and the nematodes *A. ceylanicum*, *H. bakeri* and *T. muris*. In addition, with regard to *A. ceylanicum*, *H. bakeri* and *S. mansoni*, the products were not only studied against the adult stages but also against larval stages (*A. ceylanicum* and *H. bakeri*) and *S. mansoni* schistosomula. The rationale for selecting these plants for testing against the helminths arose from their traditional uses against parasitic diseases and the anthelmintic activity of several extracts against L3 of *H. contortus* (Diehl et al. 2004; Koné et al. 2005) and *Caenorhabditis elegans* (McGaw et al. 2007; Smith et al. 2009). To our knowledge, the antischistosomal, trichuricidal, ancylostomicidal and echinostomicidal activities of most of the studied plants are reported here for the first time.

Several plant extracts showed an in vitro effect against all five trematodes and nematodes studied, which might be explained by the synergistic combination among multiple bioactive compounds contained in the crude extracts (Viljoen

Table 3 Effect on worm burden of 12 active extracts administered at a single dose of in *E. caproni*-infected (800 mg/kg) *and T. muris*-infected (400 mg/kg) mice

Plant tested	*E. caproni*			*T. muris*			
	Mean number of worms (SD)	Worm burden reduction (%)	*P* values	Mean number of worms (SD)	Worm expulsion rate (%)	Worm burden reduction (%)	*P* values
Control	29.6 (10.2)			89 (42.8)			
				125 (75.7)			
Anogeissus leiocarpus (sb)	ND			57.7 (113.8)[a]	5.2	6.2	0.436
Anogeissus leiocarpus (r)	ND			90.0 (137.8)[b]	9.4	27.7	0.436
Combretum mucronatum (l)	ND			18.3 (18.0)[b]	44.4	85.3	0.038
Crossopteryx febrifuga (r)	34.3 (5.8)	0		89.7 (171.5)[a]	3.9	3.1	0.194
Eriosema griseum (l)	ND			ND			
Erythrina senegalensis (r)	ND			ND			
Monotes kerstingii (sb)	26 (11.3)	12.2	0.46	ND			
Lannea barteri (l)	26.7 (11.0)	9.9	0.65	ND			
Olax subscorpioidea (r)	25 (3.0)	15.5	>0.99	ND			
Sacoglottis gabonensis (sb)	33.3 (9.0)	0	0.55	ND			
Securidaca longepedunculata (r)	26 (12.7)	12.2	0.44	ND			
Starchytarpheta	23.3 (5.5)	21.2	0.46				
Terminalia avicennioides (sb)	ND			52.7 (69.6)[a]	0	40.7	0.28
Xylopia aethiopica (sb)	32.3 (6.6)	0	0.46	ND			
Podophyllotoxin	ND			ND			

ND non determined, *r* roots, *l* leaves, *sb* stem bark, *w* whole plant

[a] Versus control 1

[b] Versus control 2

et al. 2005; Karmegam et al. 2008). Three compounds revealed high in vivo activities, namely *O. subscorpioidea*, *A. leiocarpus* and *C. mucronatum*. Promising antischistosomal properties were observed when *S. mansoni*-infected mice were treated with the extract of the root of *O. subscorpioidea.* The roots of *O. subscorpioidea* are used in Northern Côte d'Ivoire for treating intestinal worms (Koné et al. 2005) and malaria (Gauthier-Beguin 1992). It is interesting to note that in

Table 4 Effect on worm burden of 11 active extracts administered at a single dose of 400 mg/kg to mice harbouring adult *S. mansoni*

Plant species and control	Mean number of worms (SD)		Total worm burden reduction (%)	*P* values	Female worm burden reduction (%)	*P* values
	Total	Females				
Control	9.3 (7.5)	4.5 (4.2)	–	–	–	–
Anogeissus leiocarpus (sb)	4.0 (1.0)	1.3 (0.6)	57.0	0.44	71.1	0.49
Anogeissus leiocarpus (r)	7.8 (8.3)	4.3 (3.9)	16.1	0.44	4.5	0.17
Crossopteryx febrifuga (r)	5.0 (2.6)	1.3 (1.5)	46.3	0.49	71.1	0.26
Eriosema griseum (l)	4.7 (2.5)	2.3 (1.8)	49.5	0.55	48.9	0.55
Erythrina senegalensis (r)	14.7 (10.3)	5.7 (4.6)	0	na	0	na
*Olax subscorpioidea (*r)	3.7 (0.6)	0.7 (1.6)	60.2	0.35	84.5	0.12
Sacoglottis gabonensis (sb)	10.8 (11.7)	4.8 (5.4)	0	na	0	na
Securidaca longepedunculata (r)	10.7 (1.1)	4.3 (2.1)	0	na	4.5	na
Starchytarpheta	13.7 (9.6)	6.0 (5.6)	0	na	0	na
Xylopia aethiopica (sb)	9.0 (8.5)	4.0 (4.2)	3.2	>0.99	11.1	>0.99

r roots, *l* leaves, *sb* stem bark, *w* whole plant, *na* not applicable

particular, female schistosomes were highly affected in vivo (female worm burden reduction of 84.5%). On the other hand, no effect was observed against adult *E. caproni* in vivo, pointing to a very specific mechanism of action of this drug. The phytochemicals present in *O. subscorpioidea* are known only for the seeds of this plant, with santalbic acid being the principle component (Cantrell et al. 2003). Studies have been launched therefore in our laboratories to isolate the active components of *O. subscorpioidea* to be able to characterize the interesting antischistosomal properties in further detail. *O. subscorpioidea* has not only trematocidal but also nematocidal properties. *T. muris* were highly affected by this plant in vitro. In addition, a previous study found that *O. subscorpioidea* possessed larvicidal activity against *H. contortus* L3 (Koné et al. 2005).

The extract prepared from the stem bark of *A. leiocarpus* achieved a worm burden reduction of 71.1% against female *S. mansoni*. To our knowledge, the antischistosomal activities of *A. leiocarpus* have been described for the first time. Further studies are necessary to strengthen and confirm these positive findings, including experiments in larger animal group sizes and to also study the effect against juvenile schistosomes. Previous studies reported promising anthelmintic activities of *A. leiocarpus* against *Rhabditis pseudoelongata* (Okpekon et al. 2004) and *H. contortus* (Diehl et al. 2004; Koné et al. 2005) and antiparasitic properties against *Plasmodium falciparum* (Gansané et al. 2010). The stem bark of *A. leiocarpus* was found to contain gallic and gentisic acids (Chaabi et al. 2008; Shuaibu et al. 2008), which have recently been shown to possess anthelmintic activity (Smith et al. 2009). Studies should be undertaken to isolate the components of *A. leiocarpus* stem bark responsible for the antischistosomal activities.

High trichuricidal activity was documented for *C. mucronatum*, a plant used in Côte d'Ivoire against malaria, and in Ghana against guinea worm infestation (Ibrahim 1986). We observed a high worm burden reduction of 85.3% in vivo against *T. muris*. To our knowledge, this is the first report of the anthelmintic activity of *C. mucronatum*; its phytochemicals have not been studied to date.

Disappointingly, *E. senegalensis* lacked activity against adult *S. mansoni* in vivo despite promising activities observed against both stages in vitro. This plant is applied in traditional medicine in the treatment of urinary bilharziosis (Togola et al. 2008). The efficacy against juvenile *S. mansoni* in vivo remains to be tested. It is possible that higher worm burden reductions will be achieved against the juvenile worms in vivo, since this stage was more susceptible than the adult worms in vitro (10 versus 160 μg/ml).

In this study, we also noted an activity of *E. senegalensis* against the mouse hookworm, *H. bakeri*, however *T. muris* and *A. ceylanicum* were not affected. *E. senegalensis* also lacked activity against *H. contortus* in a previous study (Koné et al. 2005). One of the phytochemicals of *E. senegalensis* (Taylor et al. 1986; Wandji et al. 1990; Lee et al. 2009), alpinumisoflavone, was also isolated from *Milletia thonningii*, a medicinal plant used in Ghana as anthelmintic (Abbiw 1990). It has been demonstrated that alpinumisoflavone present in *M. thonningii* was highly active against *S. mansoni* miracidia, cercariae and adult worms in vitro (Perrett et al. 1995; Lyddiard and Whitfield 2001; Lyddiard et al. 2002). The presence of alpinumisoflavone in *E. senegalensis* might contribute to the in vitro activity against juvenile and adult *S. mansoni* and *H. bakeri* observed in this study.

In conclusion, the present study screened the in vitro and in vivo anthelmintic activity of 50 extracts of plant species used in Côte d'Ivoire to treat parasitic diseases. Ten of these plants exhibited significant in vitro activity against trematodes (*E. caproni* and *S. mansoni*) and nematodes (*H. bakeri* and *T. muris*), providing some validation for their traditional uses. In vivo studies showed high antischistosomal activities of *O. subscorpioidea* and high trichuricidal activity of *C. mucronatum*. For *O. subscorpioidea*, phytochemical investigations are ongoing in order to isolate the active compounds.

Acknowledgements We are grateful to the programme "Echanges Universitaires"", founded by the Swiss Agency for Development and Cooperation. JK and MV are grateful to the Swiss National Science Foundation for financial support (project no. PPOOA-114941 and PPOOP3_135170). We sincerely acknowledge the Centre Suisse de Recherches Scientifiques in Côte d'Ivoire and the students of the Helminth Drug Development Unit for technical assistance.

Conflict of interest The authors declare that they have no conflict of interest.

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