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Activity of medicinal plants from Ghana against the parasitic gut protist *Blastocystis*

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ABSTRACT

Ethnopharmacological relevance: The plants tested in this study were examples of plants historically used to treat or alleviate several types of stomach disorders manifested by *e.g.* stomachache, diarrhoea or dysentery. These plants have been consumed typically as a decoction, sometimes mixed with other flavourings. The aim of this study was to evaluate the anti-*Blastocystis* activity of 24 plant parts from 21 medicinal plants from Ghana.

Materials and methods: The medicinal plants were collected in the Greater Accra region of Ghana. Every plant part was tested in three different extracts; an ethanolic, a warm, and a cold water extract, at a final concentration of 1 mg/mL for the initial screening, and in a range from 0.0156 to 1 mg/mL for determination of inhibitory concentrations. The obligate anaerobic parasitic gut protist *Blastocystis* (subtype 4) was used as a 48 h old subcultivated isolate in the final concentration of 10^6 cells/mL. Plant extracts inoculated with *Blastocystis* were incubated at 37 °C for 24 h and 48 h. Both MIC minimum inhibitory concentration (MIC_{90}) assays and minimal lethal concentration (MLC) assays were performed after 24 h and 48 h. The half maximal inhibitory concentration (IC_{50}) was derived after 24 h and 48 h. Antimicrobial activity was tested against two Gram-positive and two Gram-negative bacteria for all 24 plant parts at a final concentration of 1 mg/mL.

Results: Screening of the 24 different plant parts showed significant anti-*Blastocystis* activity of six of the ethanolic extracts: *Mallotus oppositifolius*, IC_{50}, 24 h 27.8 μg/mL; *Vemonia colorata*, IC_{50}, 24 h 117.9 μg/mL; *Zanthoxylum zanthoxyloides*, cortex IC_{50}, 24 h 255.6 μg/mL; *Clausena anisata*, IC_{50}, 24 h 314.0 μg/mL; *Z. zanthoxyloides*, radix IC_{50}, 24 h 335.7 μg/mL and *Eythrina senegalensis*, IC_{50}, 24 h 527.6 μg/mL. The reference anti-protozoal agent metronidazole (MTZ) had an IC_{50}, 24 h of 7.6 μg/mL. Only *C. anisata* showed antimicrobial activity at a concentration of 800 μg/mL.

Conclusion: Six ethanolic plant extracts showed significant anti-parasitic activity against *Blastocystis*. *M. oppositifolius* showed nearly as good activity as the reference anti-protozoal drug MTZ. Historically, the active plants found in this study have been used against dysentery, diarrhoea or other stomach disorders. Nowadays they are not used specifically for dysentery, but they are being used as medicinal plants against various stomach disorders.

1. Introduction

The present study of anti-protozoal activity in medicinal plants from Ghana is part of a larger research collaboration investigating the use of historical and contemporary medicinal plants in Ghana (Soelberg et al., 2015). The plants tested in this study are presently used or have been used historically to treat or alleviate many types of stomach disorders manifested by *e.g.* stomachache, diarrhoea or dysentery (Petiver, 1697; Bowdich, 1819; Schumacher, 1827; Soelberg et al., 2015). Traditionally, the plants have been consumed as a decoction, sometimes mixed with other flavourings.

Parasitic infections take a toll on human health and can affect all people, not only in the tropics, but also in regions with temperate climates (Centers for Disease Control and Prevention, 2014). Some parasites such as the intestinal protozoon *Entamoeba dispar* appear harmless (Centers for Disease Control and Prevention, 2012), whereas others may cause fatal infections, for instance *Entamoeba histolytica*, a cause of dysentery, and one of the parasites causing malaria, *Plasmodium falciparum* (Centers for Disease Control and Prevention, 2010). Fortunately, most parasitic diseases are treatable with modern medicines, but several cases of resistance towards these medicines are emerging for a number of

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parasitic protists, *e.g. E. histolytica*, *Giardia lamblia* and *Blastocystis* (Debnath et al., 2014; Upcroft and Upcroft, 2001). There has been a great focus on resistance towards antibiotics, but it is clear that focus now also should be on the increasing resistance towards anti-protozoal medicines (Borst and Ouellette, 1995; Khaw and Panosian, 1995; Sinha et al., 2014).

The obligate anaerobic parasitic gut protist *Blastocystis* is an intestinal unicellular parasite of humans and a vast variety of non-human hosts. The genus exhibits extensive genetic diversity, and to date, nine ribosomal lineages (subtypes), arguably species, have been identified in humans (Alfellani et al., 2013; Clark et al., 2013; Stensvold et al., 2007). *Blastocystis* is one of the most widespread and common intestinal parasites of humans (Alfellani et al., 2012; Alfellani et al., 2013; Clark et al., 2013). It is estimated that between 1 and 2 billion people are infected with this parasite (Scanlan and Stensvold, 2013). Studies have shown a prevalence ranging from 0.5% in industrialised countries and up to 60% in developing countries. Prevalence depends on the identification technique of the parasite and can therefore vary when testing the same population. Generally, developing countries show higher prevalence figures because of poor hygiene and the ingestion of contaminated food and water (Tan, 2008).

The role of *Blastocystis* in human health and disease remains controversial (Scanlan and Stensvold, 2013). Many consider the parasite harmless due to asymptomatic carriage being common, but there is some evidence to suggest that the parasite might be related to irritable bowel syndrome and/or cause irritable bowel syndrome-like symptoms (Alfellani et al., 2012; Coyle et al., 2012; El Deeb et al., 2012; Engsbro et al., 2014; Roberts et al., 2014; Stensvold et al., 2009; Yamamoto-Furusho and Torijano-Carrera, 2010). Symptoms associated with *Blastocystis* infections are: abdominal pain, diarrhoea, vomiting, nausea, flatulence, bloating and anorexia (Roberts et al., 2014; Tan, 2008). Potential pathogenicity may be subtype-related according to several studies (*e.g.* Alfellani et al., 2012; Ramírez et al., 2014; Roberts et al., 2014; Stensvold et al., 2011).

There is no consensus regarding treatment of *Blastocystis* infection —or whether the parasite should be treated at all (Coyle et al., 2012; Engsbro et al., 2014; Roberts et al., 2014). Nonetheless, eradicating *Blastocystis* from the human intestine appears to be challenging. Metronidazole (MTZ) is the most common anti-protozoal drug of choice, even though the efficacy ranges from 0% to 100% (Stensvold et al., 2010). Resistance towards MTZ and difficulties in treating *Blastocystis* are also becoming a problem (Roberts et al., 2014; Sekar and Shanthi, 2013). The different subtypes also show different susceptibility towards antimicrobial drugs (Roberts et al., 2014). The usual adult dose of MTZ recommended to treat a *Blastocystis* infection is 500–750 mg thrice daily for 10 days or 1.5 g daily for 7 days (Sekar and Shanthi, 2013). Treatment failure may in principle be due to drug resistance, poor drug efficacy, or reinfection (Stensvold et al., 2010). Experience with alternative ways of treating an infection with *Blastocystis* is limited, but other medicines could be paromomycin, trimethroprim–sulfamethoxazole or nitazoxanide (Khaw and Panosian, 1995; Roberts et al., 2014; Sekar and Shanthi, 2013; Stensvold et al., 2010). The use of so many different compounds also indicate the challenge of eradicating *Blastocystis*.

Previous studies have shown inhibitory effect against *Blastocystis* of plant extracts of *Coptis chinensis* and *Brucea javanica* (Yang et al., 1996), *Thymus vulgaris*, *Serenoa repens*, *Vitis vinifera* and *Curcubita pepo* (Grabensteiner et al., 2008), *Allium sativum* (Yakoob et al., 2011), *Ferula asafoetida* (El Deeb et al., 2012) and *Quercus infectoria* and *Achillea millefolium* (Özbilgin et al., 2013).

The aim of the present study was to find new ways of treating infections with *Blastocystis* by using medicinal plants as a platform for the design of novel anti-protozoal drugs.

2. Materials and methods

2.1. Blastocystis

Blastocystis (subtype 4) was cultured from a faecal sample from a voluntary staff member at Statens Serum Institut, Copenhagen, Denmark. The culture, which was xenic (*i.e.* containing bacteria), used Jones medium and the isolate was propagated at 37 °C by performing subculture every two–three days.

2.2. Plant material and extracts

Medicinal plants were collected during the period November 2013–January 2014 in the Greater Accra region of Ghana. The plants were identified and authenticated by ethnobotanist Jens

Table 1
Plant species tested for anti-parasitic activity against *Blastocystis*.

Plant species	Family	Voucher number	Plant part
Boerhavia diffusa L.	Nyctaginaceae	JS 281	Herba
Clausena anisata (Willd.) Hook. f. ex Benth.	Rutaceae	JS 214R	Radix
Deinbollia pinnata Schumach. & Thonn.	Sapindaceae	JS 202	Herba
Erythrina senegalensis DC.	Fabaceae	JS 231	Cortex
Flacourtia flavescens Willd.	Salicaceae	JS 249	Folium
Flueggea virosa (Roxb. ex Willd.) Royle	Phyllantaceae	JS 252	Folium
Gardenia ternifolia Schumach. & Thonn.	Rubiaceae	JS 246	Folium
Launaea taraxacifolia (Willd.) Amin ex C.Jeffrey	Asteraceae	JS 212	Folium
Mallotus oppositifolius (Geisel.) Müll.-Arg.	Euphorbiaceae	JS 208	Herba
Newbouldia laevis (P.Beauv.) Seem.	Bignoniaceae	JS 216	Folium
Paullinia africana R.Br. ex Tedlie	Sapindaceae	JS 219	Herba
Phyllanthus amarus Schumach. & Thonn.	Phyllantaceae	JS 237	Herba con radix
Premna quadrifolia Schumach. & Thonn.	Verbenaceae	JS 283	Herba
Pupalia lappacea (L.) Juss.	Amaranthaceae	JS 239	Herba
Senna occidentalis (L.) Link	Fabaceae	JS 234H	Herba
Senna occidentalis (L.) Link	Fabaceae	JS 234R	Radix
Spathodea campanulata P.Beauv.	Bignoniaceae	JS 230	Cortex
Stylosanthes erecta P.Beauv.	Fabaceae	JS 271	Herba
Tapinanthus bangwensis (Engl. & K.Krause) Danser	Loranthaceae	JS 210	Herba
Thonningia sanguinea Vahl	Balanophoraceae	JS 296	Herba
Vernonia colorata subsp. *colorata* (Willd.) Drake	Asteraceae	JS 268FF	Folium+flos
Vernonia colorata subsp. *colorata* (Willd.) Drake	Asteraceae	JS 268R	Radix
Zanthoxylum zanthoxyloides (Lam.) Zepern. & Timler	Rutaceae	JS 243C	Cortex
Zanthoxylum zanthoxyloides (Lam.) Zepern. & Timler	Rutaceae	JS 243R	Radix

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