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Antitrypanosomal Activity of Extract Fractions of *Cetraria Islandica*

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ABSTRACT: The antitrypanosomal activity of various extract fractions of *C. islandica* (Iceland moss) was evaluated by using the well-established Alamar blue™ 96 well microplate assay. The antitrypanosomal activity significantly increased with increasing amounts of fractions (from 10-20 µg/ml) and MIC of 12.5µg/ml were observed for certain fractions. The results obtained in the present study indicate that *C. islandica* is a potential source of natural antitrypanosomal agents.

KEYWORDS: *Cetraria islandica*; Lichens; Iceland Moss; Antitrypanosomal Activity; *Trypanosoma brucei*.

INTRODUCTION

Herbal Medicine or phytomedicine involves the use of plant seeds, fruits, roots, leaves, stems, bark or flowers for medicinal purposes. The use of natural products with therapeutic properties is as ancient as human civilization [1-4]. Herbal Products are gaining attention due to the belief that they are less toxic and they possess high efficacy against free radical mediated diseases [1,5].

Cetraria islandica, commonly known as Iceland Moss is a lichen with extensive medicinal properties and traditionally used for the treatment of diseases such as hemorrhoids, bronchitis, dysentery and tuberculosis. *C. islandica* has also been shown to be a potential antioxidant, anti- H. pylori etc [6-8]. However, there is little information about the anti-trypanosomal activity of the lichen. This investigation is to determine the anti-trypanosomal activity of various fractions of *C. islandica* extracts. In addition to this, the components responsible for the anti-trypanosomal activity of *C. islandica* are presently unclear. Hence, this report could pave way for further work on the isolation and identification of the anti-trypanosomal components of *C. islandica*.

MATERIALS & METHODS

Collection of Material

The plant material was obtained from the Strathclyde Initiative for Drug Research (SIDR). Sample was air dried and coarsely powdered. A voucher specimen has been reserved in Natural Product Laboratories, SIPBS for future reference.

Preparation of Samples

The material (245.5g) was successively extracted using hexane, ethyl acetate and methanol in a Soxhlet apparatus. The crude extracts were allowed to stand and sometimes cooled to allow for the precipitation of compounds. Where all precipitates have been produced or no precipitation, the crude extracts or precipitates were subjected to column chromatography (CC) and eluted gradient-wise using hexane-ethyl acetate, ethyl acetate and subsequently ethyl acetate-methanol solvent mixtures. Similar fractions (TLC) were combined and allowed to evaporate under the fume hood.

Antitrypanosmal Activity

Samples were prepared as 10mg/ml stock solutions in 100% DMSO. These were diluted with HMI-9 medium to a concentration of 1mg/ml. 4µl of the test sample was added to the assay well then 96µl HMI-9 medium to give a final assay concentration after a 1:1 dilution of 20µg/ml. The anti-trypanosomal and cytotoxicity assays were performed using the REDOX indicator Alamar blue™. The active component of which is resazurin (blue in colour) which in the presence of live parasites or cells is reduced to the bright pink fluorescent resorufin. The fluorescence values for the test plates are measured using a microplate reader in fluorescence mode with excitation and emission wavelengths of 560 and 590nm respectively. Wells containing active compounds are easily identified as they remain blue in colour and have background levels of fluorescence. The test samples are initially screened at a single concentration and then MIC or IC_{50} values are determined for the active compounds at n=2 or n=3. Positive and negative controls and a sterility checks are included in all assays. The incubation and treatment times, incubation conditions and seeding densities are optimised for each test species and cell line. Samples were initially screened at a single concentration usually 20µg/ml for extracts. The concentration of dimethylsulphoxide (DMSO) should not exceed 0.5% in the initial screen[9].

RESULTS & DISCUSSION

Table 1 Results of the antitrypansomal screening of *Cetraria islandica*, A lichen. Key: ND = Not done; H = Hexane; E = Ethyl acetate

S. No	Ref code	Fraction No.	Solvent system	% D control 20ug/ml T. brucei	% D control 10ug/ml T. brucei	MIC (µg/ml) T. brucei
1	C 1-7	A	H:E 80:20	-------	---------	ND
2	C 14-18	B	H:E 80:20	0.4	0.6	12.5
3	C 19-21	C	H:E 80:20	0.7	10.7	12.5
4	C 22-27	D	H:E 80:20	0.2	18.9	12.5
5	C 28-35	E	H:E 80:20	0.7	4.4	12.5
6	C 36-38	F	H:E 20:80	27.0	95.8	ND
7	C 39-42	G	H:E 20:80	106.5	108.9	ND
8	C 43-50	H	E:M 50:50	101.8	102.4	ND
9	Suramin					10

Various fractions of *Cetraria islandica* were obtained and coded A-H. All the fractions (Samples) were evaluated for their growth inhibitory action against *Trypanosoma brucei* at two concentrations: 10 and 20 µg/ml (**Table 1**). Fraction B, C, D and E were also evaluated for their MIC values (µg/ml) against *T. brucei*. Results revealed that Fraction A was inactive at these concentrations, while fractions B-E showed potential anti-trypanosomal activity with MIC values of 12.5µg/ml. This study leads to the conclusion that *C. islandica* could be incorporated in to modern medicines for the treatment of trypanosomiasis. Further isolation and characterization of the constituents responsible for the observed activity is recommended.

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