

Bioassay-guided isolation and purification of phytochemicals with anti-parasitic properties from *Cissus ruspolti*

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Many smallholder farmers and pastoralists rely on their indigenous knowledge, practices and locally available plants to manage both human and livestock parasitic infections⁽¹⁾. We previously identified in screening study that aqueous crude extract and its relatively late-eluting fractions from one of such plants, *Cissus ruspolti*, possess strong *in vitro* anthelmintic properties^(2,3). Thus, these fractions may contain phytochemicals with anti-parasitic properties. The objective of this study was to further isolate, purify and characterise anthelmintic phytochemicals from *C. ruspolti*.

A crude aqueous extract of *C. ruspolti* was prepared by maceration in de-ionized water for 72 hr at room temperature. This extract was fractionated on a Bio-Gel P-2 column chromatography using de-ionized water and fractions were bio-assayed in triplicate for egg hatch inhibition (EHI) using *Teladorsagia circumcincta* eggs as per guidelines for anthelmintic resistance testing⁽⁴⁾ to locate bio-active pools. Isolation and purification of phytochemicals from one of the active pools was carried out through Sephadex LH-20 column chromatography by solvent gradient elution, and were also bio-assayed in triplicate, using the chemical anthelmintic monepantel (Zolvix[®]; 0.1µg/mL) as a positive control. Further characterization of compounds was done using thin-layer chromatography (TLC) developed in BuOH: HOAc: H₂O (4:1:1) and CHCl₃: MeOH: HOAc (10:2:1) solvent systems with markers, stained with vanillin-H₂SO₄ and heated in an oven at 120°C for 30 minutes.

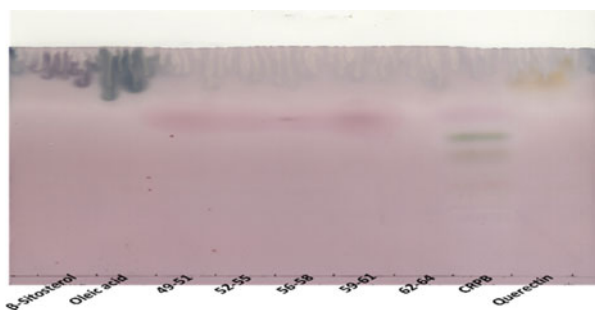


Fig. 1. EHI assays of purified *C. ruspolti* compounds

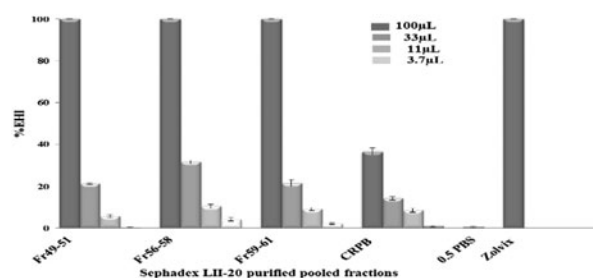


Fig. 2. TLC profile in BuOH: HOAc: H₂O (4:1:1)

We observed highly significant and dose dependent EHI activities of three pools from the Bio-Gel P-2 fractionation (data not shown). The Sephadex LH-20 purified active compounds, which also showed strong and dose-dependent EHI activities (Figure 1); the highest concentration used for some of the components resulted in 100 % EHI. The TLC analyses of these pure compounds indicated that they are polar compounds with higher average retention factor ($R_f = 0.553$) in polar and acidic solvent system (Figure 2) than in relatively non-polar and acidic solvent system: chloroform- methanol-acetic acid ($R_f = 0.194$; data not shown).

Our data support the view that *C. ruspolti* has anti-parasitic properties and also validated its ethno-medical use. Moreover, these *in vitro* experiments provided preliminary screenings for efficacy of extracts, fractions and purified compounds. Purified samples obtained have been submitted for NMR and MS analysis for structure elucidation, which is expected to inform pending *in vivo* trials, underpin existing phytotherapy and potentially the development of novel anthelmintic drugs.

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