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ANTI-TRYPANOSOMAL ACTIVITY OF HPLC PURIFIED PRECOCENE I FROM AGERATUM HOUSTONIANUM LEAVES AGAINST TRYPANOSOMA EVANSI

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Freshly harvested leaves of *Ageratum houstonianum* were dried under shade and powdered. Leaf sample of A. *houstonianum* was extracted by process of hydrodistillation using a Clevenger-type apparatus for the preparation of essential oil. Extract from A. *houstonianum* was prepared by dissolving 5 µL of the essential oil in 10 mL methanol. All the sample was filtered through a Whatman (Maidstone, England) stainless steel syringe assembly using a 0.22 µm Durapore (Millipore: Milford, USA) membrane filter. HPLC analysis was carried out via a Waters HPLC system consisting of model 510 and 515 pumps, a Rheodyne injector, a Novapak C18 column (250 x 4.6 mm i.d.; 4 µm), a model 490E multi-channel detector and Millennium 2010 sata manager. The mobile phase constituents were filtered using a Durapore 0.22 µm membrane filter. The elution was carried out with a linear gradient of acetonitrile: water (40:60) to pure acetonitrile in 60 min at a flow rate of 1 mL/min. detection was at 210, 240, 280 and 320 nm. The precocene was eluted within 25 min, the peak areas showed good reproducibility (average relative standard deviation were 0.78%), and the calibration curves (i.e. mass of precocene standard injected vs. peak area detected at 210 nm) were linear over the range of 0.05-10 µg (for precocene I, y = 6654454 x + 176626, r2 = 0.99 and for precocene II, y = 4618457 x + 133472, r2=0.99). Standard sample containing precocene I (1 mg/mL) and precocene II (1 mg/mL) obtained from Sigma (St Louis, MO, USA) were prepared in methanol. Identified precocene I was screened against *Trypanosoma evansi* for trypanocidal activity on Vero cells grown in Dulbecco's Modified Eagle Medium (DMEM) and supplemented with foetal calf serum (FCS) 20-40% at appropriate conditions. *In vitro* cytotoxicity test of precocene I at concentrations (1.56–100 µg ml-1) was done on Vero cells but without FCS. *In vitro* trypanocidal activity varied from immobilization, reduction and to the killing of trypanosomes in corresponding ELISA plate wells. At 250 µg ml-1of purified precocene I, there was drastic reduction of average mean trypanosomes count to complete killing of trypanosomes (40.±0.0 to 0.00±0.00) at 9 h of incubation, which was statistically the same as diminazine aceturate (50 µg ml-1) at 4 h. Trypanosomes counts decreased in concentration and time –dependent manner with significant difference (P≤0.05 to 0.01)). During *in vitro* cytotoxicity test, Purified precocene I and diminazine aceturate standard drug, were cytotoxic to Vero cells at all concentrations except at concentrations of 6.25–1.56 µg ml-1 and 1.56 µg ml-1, respectively. Precocene I was responsible for higher anti-trypanosomal activity. Precocene I could be the near future trypanocidal compound for a new trypanocide.

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