Essential oil components of *Hypericum androsaemum* infusions and their nematotoxic effects against *Meloidogyne javanica* (Treub) Chittwood.

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Hypericum androsaemum L. is one of the most demanded plant species in the Portuguese popular medicine being associated to the natural History of the Gerês mountain ridge (Northern of Portugal), due to its ethnobotanical uses given by the local people [6]. Traditionally, *H. androsaemum* is sold dried, under the designation of "hipericão do Gerês" and is used as an infusion [7], in cases of nephritic colic impaired digestion, boredom, and liver disturbances. The essential oils of *Hypericum androsaemum* L. were isolated by separated hydrodistillation of its leaves, stems and ripened seed capsules and by pentane extraction of the respective leaves infusion. Subsamples of fresh leaves (10g), stems (10g) and ripened seed capsules (10g) were hydrodistilled with 500 ml of boiling water in a Clevenger type apparatus over 1h, using volumes of 1.0 mL of *n*-hexane, containing 5-α-cholestane (1mg/mL), for retention of the hydrodistillate components. The infusion was prepared by adding 150 ml of boiling water onto 15 g of the dried plant leaves keeping it submersed during 5 minutes. The infusion was left to cool for 15 minutes and then it was filtered and the respective essential oil constituents were extracted with 5 ml of n-pentane containing 5-cholestane (0.1 mg/ml). The hydrodistillates and n-pentane fraction of the leaves infusion extract were analysed by GC and GC-MS in order to identify and quantify the respective components. The EO yield of leaves was 0.85 mg/g of biomass dry weight distributed in 103 compounds, 82% of which were identified. From the 92 compounds detected in the EO of ripened seed capsules, 96% were identified. The EO from stems, with only 63 compounds identified, had a narrower range of compounds compared to leaves and ripened seed capsules. From the 56 organic compounds found in the n-pentane extract from the leave infusions, 45 were identified, as leaves essential oil constituents.

Eggs of *Meloidogyne javanica*, obtained directly from egg masses isolated from the tomato infected roots, were used for hatching studies. The egg masses were placed in a 5 cm-diam. Petri dish with distilled water and then, all eggs containing juveniles in the same second stage (J2), were separated under a stereomicroscope and transferred (20/ well) one by one or in small amounts with the aid of a dissection needle and/or a feather-stitch, to each of the wells of Enzyme Linked Immuno Sorbent Assay - ELISA (6 wells) plates. All tests were conducted in the wells of the ELISA plates containing 5 ml of the *Hypericum* water extracts at different concentrations: 4, 6, 8 and 10 g/100 ml. Distilled water was used as control with five replicates of each extract concentration. The plates were placed in the dark at 22 ± 2 ºC. Cumulative juveniles eclosion was calculated separating, and adding to the anterior ones, the juveniles ecloded from the eggs, every 24 hours till 360 h. Homogeneity of variance was verified for cumulative J2 eclosion by Bartlett's test. When it was not verified, data were transformed to log 10 (x+1) prior to ANOVA. Significant differences were analyzed by Tukey's or Dunn's tests ($P < 0.05$). For mortality studies egg masses of *M. javanica* were placed on a small square piece of muslin with 30 µm openings supported as a small sieve (2.5 cm ∅, 1 cm high) in a Syracuse (2 ml cap.) glass block; about 1 ml tap water was added, until the muslin was just submerged. Juveniles collected during the first 24 hr were discarded and the subsequent J2 collected were used. Twenty J2 were exposed to 2 ml of each of the same as anterior concentrations of the lyophilized extract: 4, 6, 8 and 10 g/100 ml. As before, distilled water served as control with five replicates of each extract concentration and the tests were conducted in the wells of ELISA plates maintained in the dark at 22 ± 2 ºC. A relation between the concentration of the extract and hatching of *M. javanica* was found. The effect on eclosion inhibition was higher for 10 mg/ml than for 4 mg/ml. However, in the first 24 h apparently there was no eclosion. The mortality of J2 of *M. javanica* was directly dependent on the concentration of the extract. being observed in the first 24 hours for 6, 8 and 10 mg/ml but only after 72 h with 4 mg/ml.

Acknowledgements: This work was supported by the HypericumBiotech project, Ref. PTDC/AGR-AAM/70418/2006)

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