

## ABSTRACT

The aim of this research project was to study the chemical composition and biological activities of four species of the genus *Hypericum*: *H. perforatum* L., *H. connatum* Lam, *H. hircinum* L., *H. perforatum* L.

The isoflavone genistein, identified by NMR spectroscopy, was purified by chromatographic methods from an ethanol extract of *Hypericum connatum*.

The polyphenolic composition of an ethanol and an ethyl acetate extracts of *H. connatum* was studied. Even if the two extracts exhibited a similar polyphenol amount, UPLC analyses revealed a different profile, with a presence of rutin and apigenin in the ethanol extract, and of caffeic acid, epicatechin, *p*-coumaric acid in the ethyl acetate extract.

The scavenging activity of the extracts of *H. connatum* was evaluated by DPPH radical method. The ethanol extract showed antioxidant activity twice that of ethyl acetate, probably due to the presence of the flavonoid rutin.

The chemical composition of the essential oils of *H. perforatum*, *H. perforatum* and *H. hircinum* was analyzed by GC and GC/MS. In the three oils a total of 111 compounds were identified: 53 for the essential oil of *H. hircinum* (93.7% total oil), 55 for *H. perforatum* (96.5% total oil) and 63 for *H. perforatum* (98.7% total oil). The major fraction of the essential oils of *H. hircinum* and *H. perforatum* was constituted by sesquiterpenes while, the monoterpene,  $\alpha$ -pinene, and the phenol, thymol, were the most abundant components in the essential oil of *H. perforatum*.

The oils were also evaluated for their potential *in vitro* phytotoxic activity against germination and early radicle elongation of *Raphanus sativus* and *Lepidium sativum*. The germination of this latter was significantly inhibited by the essential oil of *H. hircinum*, at the highest doses tested, whereas radicle elongation of garden cress was significantly inhibited by the essential oils of *H. perforatum* and *H. hircinum*. The radicle elongation of radish was inhibited by the essential oil of *H. hircinum*, in major extent, and by *H. perforatum* and *H. perforatum*, in minor measure.

Another study, conducted by the escape test, was carried out in two different periods: firstly it was evaluated the protective effect of acute and chronic administration of the plants about the behavior consequences in rats induced by unavoidable stress; in the second period it was evaluated the protective effect of two chromatographic fractions obtained from the ethanol extract of *H. connatum*. The rats were pre-tested and tested after acute or chronic treatment. In particular, from the data obtained after the acute treatment, the ethanol extract of *H. connatum* was significantly active at both doses tested (0.5 g/kg pv os, 1 g/kg pv os), compared to controls. Furthermore, the extract of *H. perforatum* was active at a dose of 0.5 g/kg pv os; while the extract of *H. perforatum* was active at the dose of 1 g/kg pv os. However, the extract of *H. hircinum* was showed no significant effects. From the data obtained for repeated treatment, at the dose of 1 g/kg pv administered orally twice a day for 14 days, all the extracts tested were significantly active, compared to controls. From the data processed in the second period, after acute treatment, both chromatographic fractions of *H. connatum* administered a dose of 0.250 g/kg pv os were significantly active. Subsequently, from the chromatographic fraction C three compounds were purified by HPLC and identified by NMR methods: isoquercetin, rutin and quercetin.

Moreover, the antimicrobial activity of the three *Hypericum* species (*H.*

*connatum*, *H. hircinum*, *H. perfoliatum*) against two pathogenic emerging microorganisms (*Chromobacterium violaceum* and *Cronobacter sakazakii*) was verified. Particularly, the ethanol extract of *H. connatum* and the extract petrol of *H. hircinum* were significantly active against *C. violaceum*, while, both petrol extracts, chloroform and chloroform: methanol (9:1) extracts of *H. perfoliatum*; petrol and chloroform extracts of *H. hircinum* were significantly active against *C. sakazakii*.

For the first time, the activity anti-quorum sensing of extracts of *H. connatum* against strains of *C. violaceum* was studied. In particular, the ethanol extract, at a dose of 25 µg, inhibited the quorum sensing-regulated violacein pigment production, in *C. violaceum* tester strain, without interfering with its growth.

Finally, the presence of cardenolide glycosides in the ethanol extract of *H. connatum* has been demonstrated by chemical test. It has been also verified the cardiotoxic activity of the ethanol extract of *H. connatum*.