ANTIBACTERIAL AND ANTIPROTOZOAL EFFECT OF ARTEMISIA ANNUA EXTRACTS

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Introduction

Artemisia annua (Asteraceae) is an annual aromatic herb native to Asia that is cultivated in the whole world. A. annua contains the sesquiterpenelactone artemisinin that is used in the treatment of human malaria caused by the protozoan parasite Plasmodium falciparum, furthermore it has a high content of other bioactive secondary metabolites. Two of the most common infections in poultry, are blackhead, caused by the protozoan parasite Histomonas meleagridis (HM), and necrotic enteritis (NE) caused by the bacteria Clostridium perfringens (CP). At present there is no treatment of blackhead disease, and the preventive treatment towards NE may soon be banned in the EU, and alternatives are therefore needed.

In the work presented here, the focus was:
1) Isolation of antibacterial compounds from A. Annua extracts.
2) Determination of the minimal inhibitory concentration (MIC) values of the fractions and pure compounds from A. Annua extracts for use on CP.
3) Determination of the antiprotozoal effect against HM of the extracts.

Extraction, isolation and identification

Aerial parts of Artemisia annua were extracted with hexane or dichloromethane overnight. The dried extract were tested in a CP bioassay and activity were confirmed (marked with green in table 1). Hereafter the extracts were subjected to bioassay guided fractionation with flash column chromatography, using hexane/ethyl acetate gradient followed by isocratic step with 20 % methanol in dichloromethane. This resulted in 16 fractions and 8 fractions for the hexane and dichloromethane extract respectively. Several fractions were active, LC-MS revealed that ponticaepoxide where the major metabolite in 3 of the active fractions (MIC = 150-300 ppm) (marked with dark blue in table 1), another active fraction contained mainly scopoletin, casticin and chrysosplenol (MIC = 300 ppm) (marked with yellow in table 1) and the last selected fraction with medium activity contained 2,4-dihydroxy-6-methoxyacetophenone (2,4dh-6-m) (MIC = 600 ppm) as the only major component. All 5 of theses compounds were isolated and purified by RP prep. HPLC with an acetonitrile-water gradient and the compounds were identified by LC-MS and NMR. The MIC of hexane and DCM extracts, fractions and purified compounds were determined in a CP bioassay see table 1, fractions and scopoletin that did not yield in any antibacterial activity is not shown.

Antibacterial and antiprotozoal tests of extracts, fractions and pure compounds

Antibacterial tests was performed in overnight cultures of CP strains, isolated from diseased broilers and MIC were determined.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>MIC (ppm)</th>
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<tr>
<td>DCM extract</td>
<td>F2 DCM</td>
</tr>
<tr>
<td>ponticaepoxide</td>
<td>chrysosplenol-D</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 MIC of extracts, fractions and pure compounds. *MIC not determined for these compounds but slight inhibition seen at 800 ppm.

The highest antibacterial effect was observed in fractions containing the polyacetylen ponticaepoxide. Of the purified compounds ponticaepoxide also shows the highest antibacterial effect (MIC = 200 ppm). The hexane extract showed higher antibacterial effect than ponticaepoxide, it contains other antibacterial compounds than ponticaepoxide and compounds that may have a synergistic effect on ponticaepoxide

The antibacterial effect was tested in vivo, the hexane extract were incorporated in the diet of broilers, applied a NE disease model, the treatment reduced the population of CP and the severity of the associated small intestinal lesions (P>0.05).

HEX, DCM and artemisinin were also tested against the parasite HM. The two latter showed highest antiprotozoal effect in vitro (MLC=1.0 mg/ml and IC50=1.3 mg/ml respectively), and were tested in vivo in HM infected poultry. However, no effect against HM at the given concentrations was observed.

Conclusions and Perspectives

The tested compounds and extracts are far from as effective as the antibiotics used in the poultry industry today but ponticaepoxide has not before been show to be antibacterial and could be a potential drug lead against CP. Chrysosplenol-D also showed activity against CP, but the activity of the fraction containing chrysosplenol together with other metabolites was higher. The DCM extract and artemisinin showed activity against HM in vitro but not in vivo, and investigation of the bioavailability of artemisinin will be conducted to determine the uptake of artemisinin in poultry.

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