Evaluation of anti-microbial potential of hydroxycoumarins

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Introduction

Coumarins comprise a very large class of substances found in plants and are made of fused benzene and α-pyrone rings. More than 1300 coumarins have been identified, principally as secondary metabolites in green plants but also in fungi and bacteria. Coumarin and its derivatives are considered nowadays an important class of organic compounds due to their wide spectrum of biological activities and therapeutic applications, including anticoagulant, estrogenic, dermal photosensitising, vasodilator, molluscucidal, antihelmintic, sedative and hypnotic, analgesic and hypothermic activity. [1,2] A number of studies have reported anti-microbial activities of various coumarins. In contrast, there are few reports of anti-microbial potential of hydroxycoumarins. In this contest, six hydroxycoumarins (Figure 1) were tested. The anti-microbial properties were evaluated by two different methods using *Fusarium oxysporum* (CCMI866), *Aspergillus niger* (CCMI296), *Cladosporium* 7F1 and *Trichoderma* (CCMI783) to access the antimicrobial spectrum of active compounds.

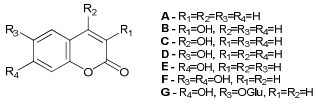
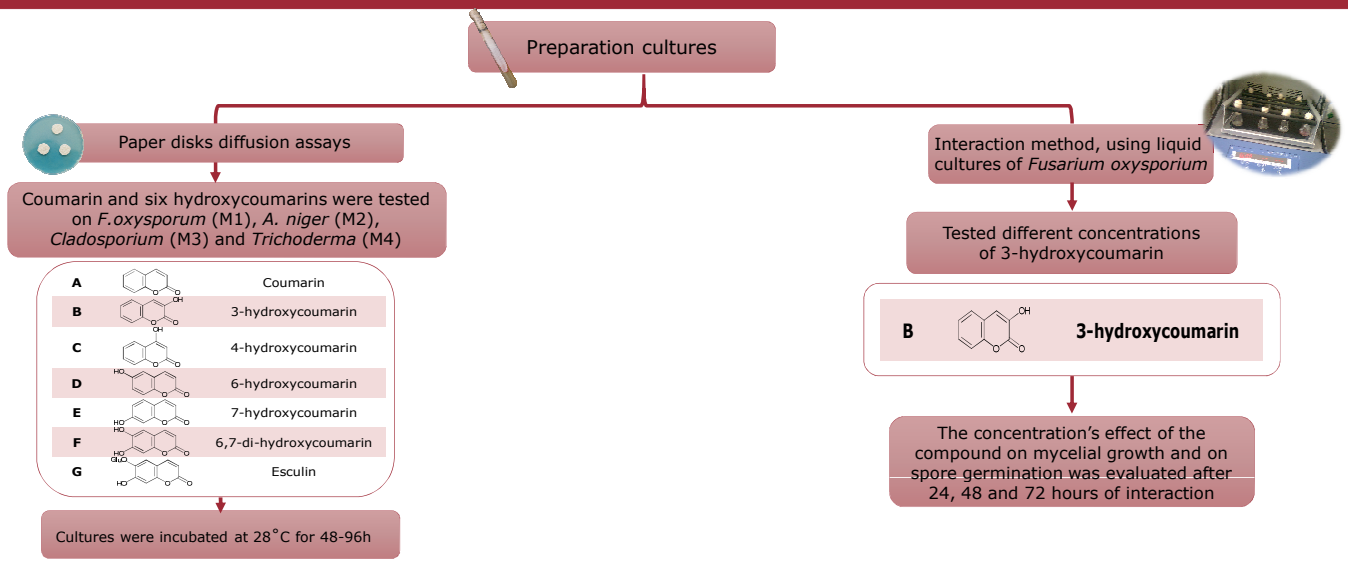


Figure 1

Materials and Methods



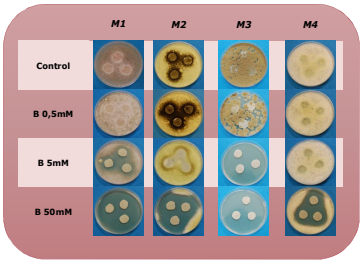
Results and Discussion

1. Screening of active compounds

	M1	M2	M3	M4
A	–	–	±	–
B	++++	+++	+	+
C	–	+	±	–
D	–	–	–	–
E	–	+	±	–
F	–	–	±	–
G	–	–	–	–

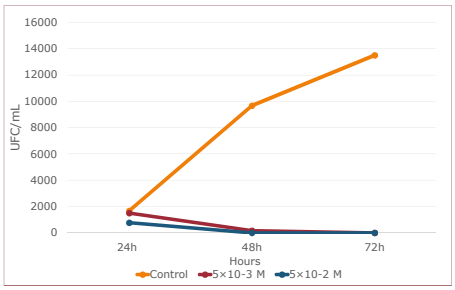
× The table depicts a qualitative measure of inhibition per compound (A,B,C,D,E,F,G) and fungus (M1, M2, M3 and M4).
× Inhibitory activity of the compounds is classified as follows: - no inhibition, ± very low inhibition, + low inhibition, + + moderate inhibition , +++ high inhibition, + + + + complete inhibition.
× More active compounds – **3-hydroxycoumarin (B)**

2. Anti-fungal activity of 3-hydroxycoumaryn



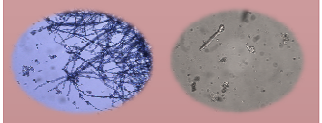
× The table shows images of inhibition halos for compound B at different concentrations (0.5 mM, 5 mM and 50 mM)

3. Dynamic interaction of 3-hydroxycoumarin and *Fusarium oxiporium*



× 3-hydroxycoumarin (B), was tested at concentrations 5,0 mM and 50,0 mM in an interaction method, using liquid cultures of *F.oxysporum*.
× The dynamic interaction of 3-hydroxycoumarin and *F.oxysporum* during 72h, shows total inhibition at the end of the assay.

Microscopic features of interaction assay



Microscopic features of the interaction assay between *F. oxysporum* and 3-hydroxycoumarin.
A – control assay, showing well developed hyphaes with membrane integrity; **B** – interaction assay after 72h, revelling fragmented hyphaes and no germinated spores.

Conclusions

× Results demonstrate that the presence of hydroxyl group in the α-pyrone is essential for an effective anti-fungal activity.
× The higher activity revealed by the 3-hydroxycoumarin (B) suggests that the electron delocalization induced by benzene ring affects directly the hydroxyl groups, causing such differences in activity.
× The results clearly show a decrease of spore germination of *F. oxysporum* and a reduced mycelial growth after 48 hours of interaction, and after 72 hours a total inhibition of growth was observed in both tested concentrations.

References

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