Novel Anti-inflammatory drug discovery from insect-infecting fungi

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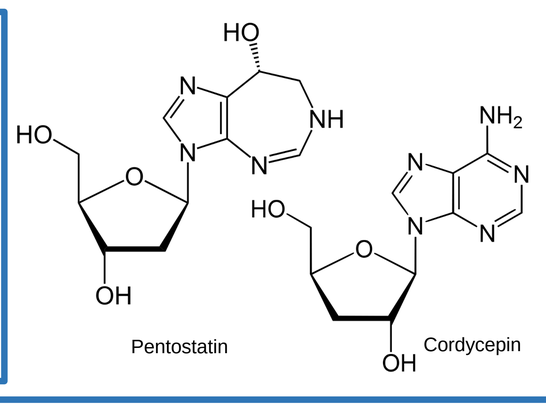
INTRODUCTION

Cordyceps militaris is an entomopathogenic fungus contains a wide range of active metabolites. Most significant metabolites isolated are cordycepin [1], pentostatin [2] an anti-inflammatory drug used in far eastern and Chinese medicine [3][4].

Cordycepin and pentostatin are adenosine analogues with similar bioactivity profile, which mimic adenosine like activity and can inhibit adenosine dependent processes [5]. Cordycepin shows to inhabit the RNA synthesis using mTOR and AMPK signal transduction, protein synthesis, and inhibition of polyadenylation pathways. It inhabit the inflammatory gene expression [6]. Pentostatin is an irreversible adenosine deaminase inhibitor, it safeguard cordycepin and prevents the deamination of cordycepin to 3'-deoxyinosine [2].

Secondary metabolites are responsible for the bioactivity, variation in secondary metabolites can cause variability in the bio-activity. Metabolic profiling is essential step for product standardization [7], it will demonstrate the quantity of the compounds of interest and will give us complete picture of potential important secondary metabolites



Methods

C. Militaris strain (Mycomedica, Solvenia) cultured on grains.

- solvent insertion was used for sample preparation and dried with speedvac obtained dried sample.
- Dried sample used for RT-qPCR analysis using RAW-264.7 cells line.
- LC-MS analysis was used for the untargeted and targeted metabolomics analysis.

LC-MS Method

Sample prepration

- Macerating powdered *Cordyceps* in water-ethanol (40:60), at 1:20 in solvent [8]

LC-MS Setup

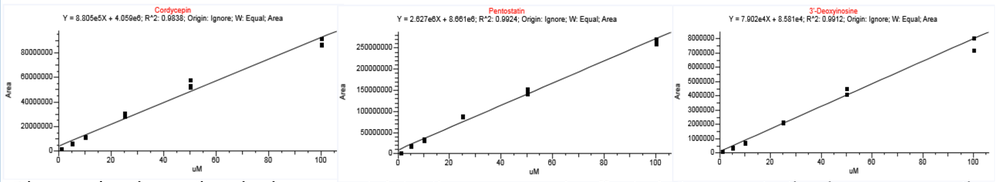
- LC: Accella® Autosampler (HPLC) and ZIC® -pHILIC column (mobile phase = 20mM $(NH_4)_2CO_3$, stationary phase = C_2H_3N)
- MS: ESI ion source, Exactive® Orbitrap
- Column performance assessed using QC sample interspaced between each 5 samples [9]

Untargeted Run: Mixture of standards A,B,C,D,E [13]
Targeted Run: Cordycepin standards: 1,5,10,25,50,100uM

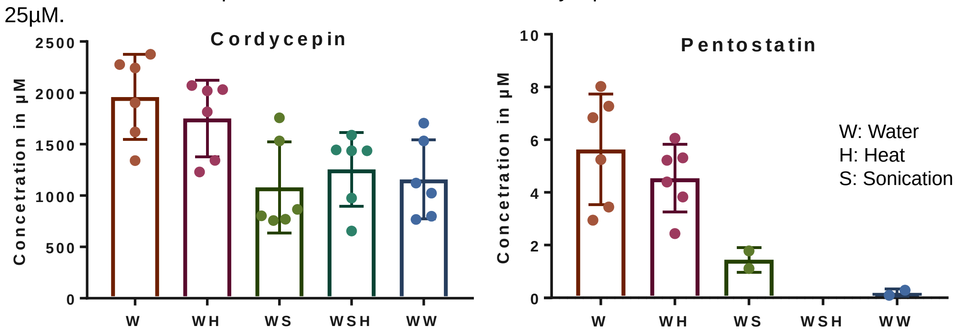
Data Analysis Software

- Untargeted: XCMS [10], mzMATCH [11], and IDEOM [12]
- Targeted: Tracefinder

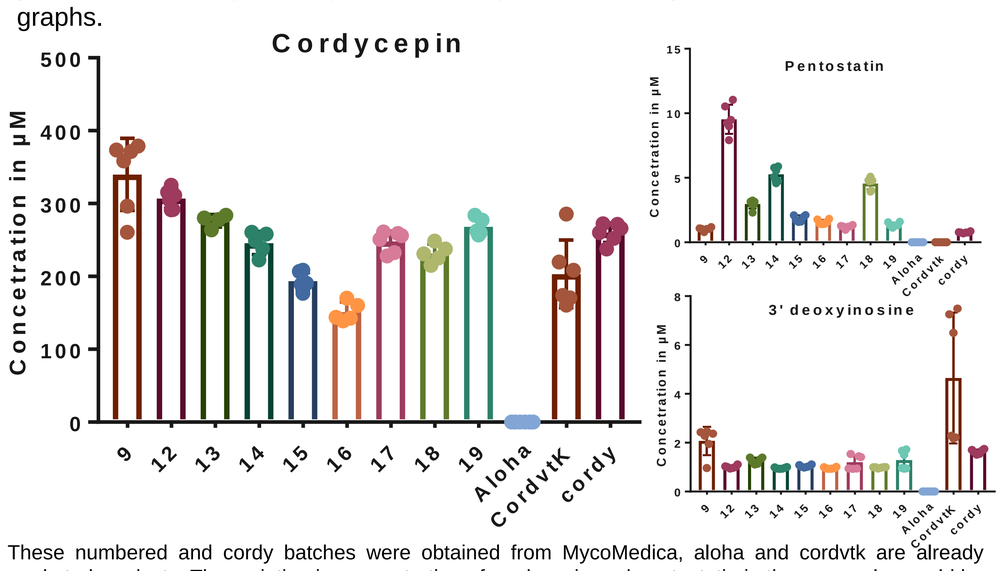
LC-MS targeted Analysis Results



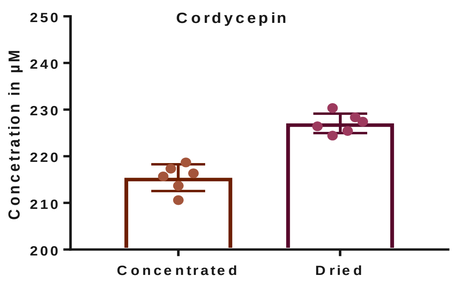
The results shows that the instrument detection accuracy is effected at concentration increases, as the increase in the area is parallel to the concentration of cordycepin until it reaches the concentration of 25µM.



The results shows that parameters such as heat, sonication and maceration time had no significant effect on increasing cordycepin concentration while the concentration of pentostatin was significantly decreased by all the three parameters as shown in the graphs.



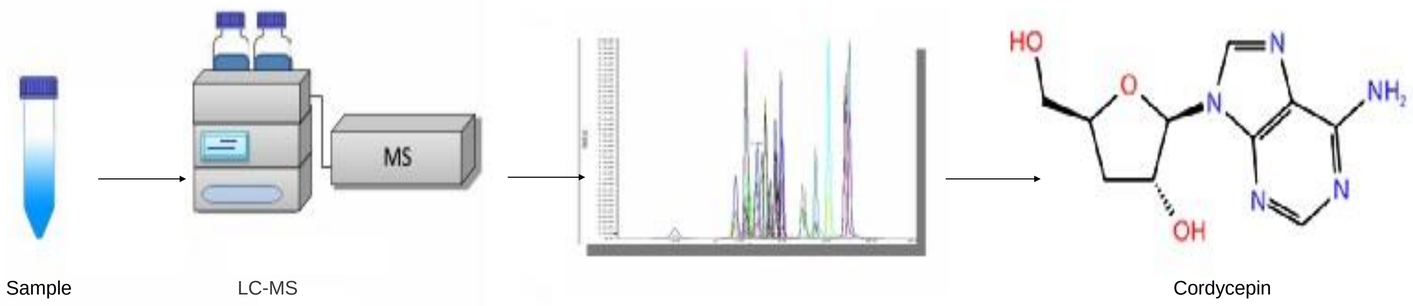
These numbered and cordy batches were obtained from MycoMedica, aloha and cordvtk are already marketed products. The variation in concentration of cordycepin and pentostatin in these samples could be due to different factory such as strain, substrate and drying process. Extraction solvents and parameter were kept constant.



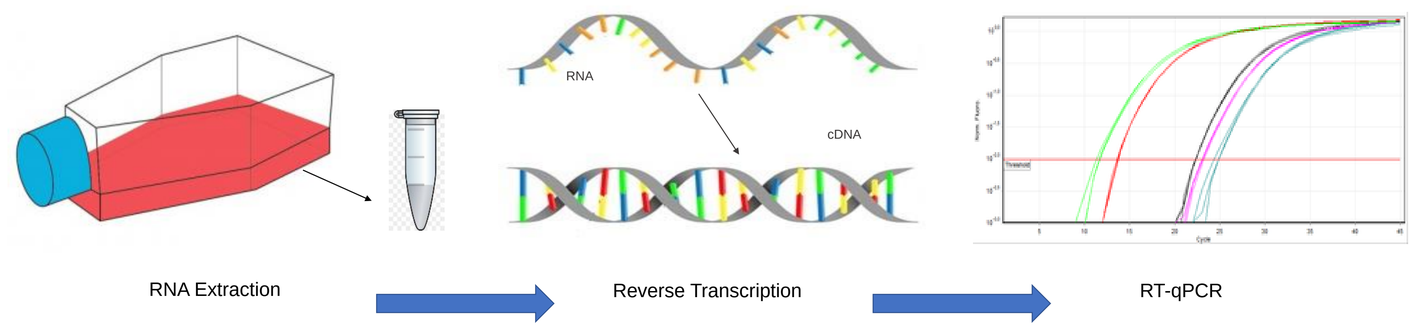
LC-MS Untargeted Analysis

Dried extract of ethanol-water solvent system showed the best extraction results according to the number and quantity of metabolites while keeping all parameters constant for both samples. Dried sample is more stable easy to handle and store.

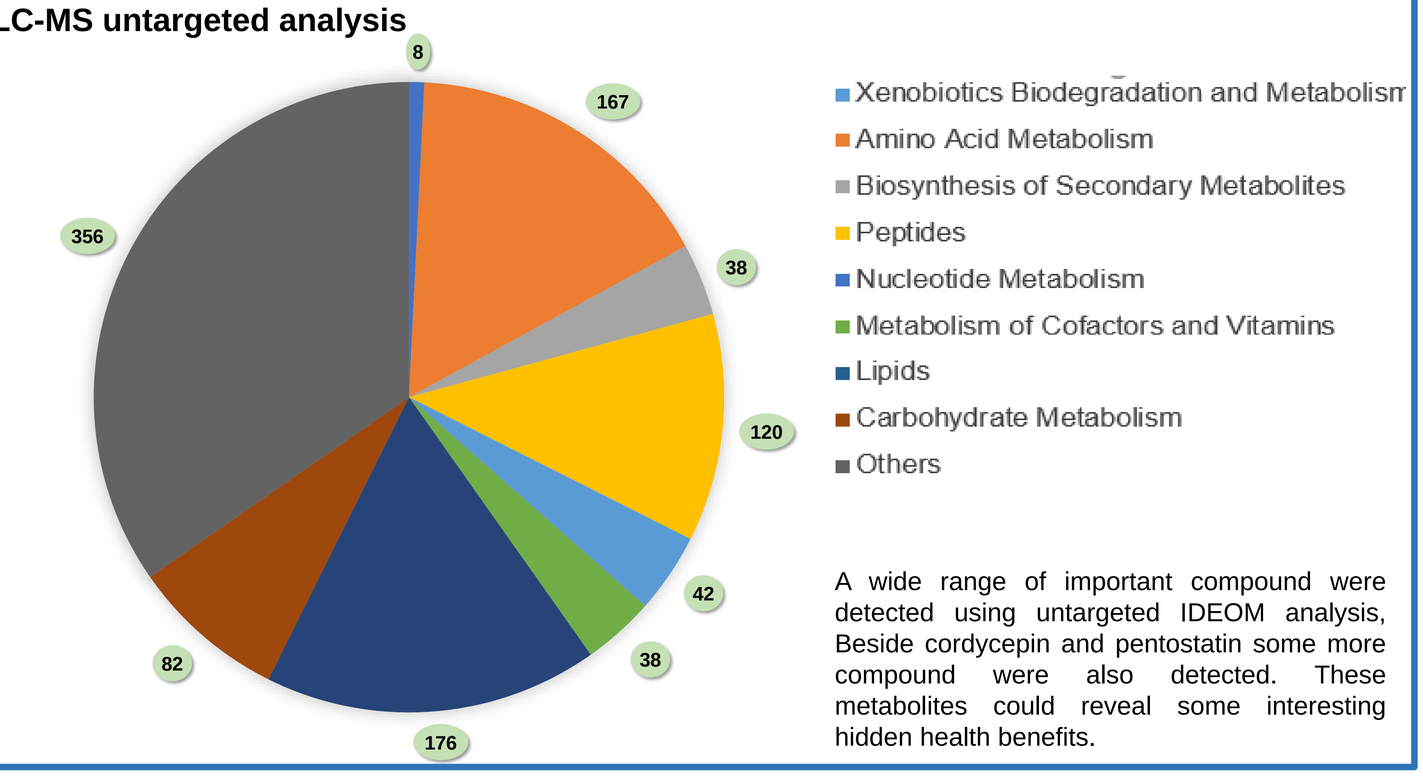
Schematic diagram of LC-MS method



Schematic diagram of RT-qPCR method



RT-qPCR Results



Tnf Inflammatory gene suppression by RT-qPCR. Error bars indicates 95% confidence with p values indicated by: *=p<0.0417, **=p<0.0010,****=p<0.0001, as calculated by t-test with Dunnett's multiple comparisons.

The RT-qPCR results show an interesting pattern of inflammatory gene repression in RAW 264.7 macrophage cell lines, indicating the anti-inflammatory activity in our sample. Pentostatin enhanced the activity of the extracts significantly, while alone showed no effect; it could be because of the synergistic effect with Cordycepin. The active sample was tested with higher concentration, which shows that the activity of our sample increased which indicates dose dependence.

LC-MS untargeted analysis

A wide range of important compound were detected using untargeted IDEOM analysis, Beside cordycepin and pentostatin some more compound were also detected. These metabolites could reveal some interesting hidden health benefits.

Conclusion

Cordyceps militaris activity as anti-inflammatory has been shown by the RT-qPCR and LC-MS results confirms the presence of abundant secondary metabolites (Cordycepin, pentostatin, adenosine), which are reported for anti-inflammatory activity. Pentostatin safeguard cordycepin from degradation and it enhances its anti-inflammatory activity [14]. The LC-MS results also show the presence of numerous different metabolites which could responsible for several activities. RT-qPCR and LC-MS results shows that macerating powdered *Cordyceps* at room temperature results in stable and more potent extract. Ethanol and water was best solvent system to obtain a potent bioactive extract. The results also show that of extracts does not effected the concertation and number of metabolites significantly.

1. Ni, H., Zhou. *et al.*(2009) *J. Chromatography 22:* 2135-2141; 2. Tuli, H. S., Sharma *et al* (2013) *Life sciences* 23: 863-869; 3. Cunningham, K.G. et al. (1950) *Nature* 166: 949; 4. Won, So-Young *et al* (2005) *J. ethnopharmacology* 555-56; 5. Adamson *et.al.* (1977) *Pharmacology* 15: 1 84-89; 6. Kondrashov *et. al.* (2012) *Rna*; 7. Lazarowych *et.al.* (1998) *Drug info J* 32: 497-512; 8. Wang .H. J *et.al* (2014) *Molecules* 20808-20820; 9.Want, E.J. *et al.* (2010) *Nature Prot.* 5: 1005-1018; 10. Tautenhahn, R. *et al.* (2008) *BMC Bioinf.* 11: 500; **9.**Scheltema, R.A. *et al.* (2011) *Anal. Chem* 83: 2786-2793; **12.**Creek, D.J. *et al.* (2012) *Bioinf.* 28: 1048-1049; 13. Schatschneider, S. *et al.* (2012) *Anal Chem*90: 4470-4477; 14. Xia Y *et. al* (2017) *Cell Chem Biol*1479-1489.