7-2013

Anti-TB and Antibacterial Activities of Natural Products Extracts

Douglas Armstrong
Olivet Nazarene University, darmstrg@olivet.edu

Nathan Krause
Olivet Nazarene University

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Dr. Douglas Armstrong and Nathan Krause
Olivet Nazarene University
Bourbonnais, IL 60914

Gordon Research Conference, July 28 - August 2, 2013

Introduction

In a general way, bioactive natural products are dealt with very well by Liang & Fang, 2006 (See "References" section below.) More specifically, the southwestern part of the United States has a large variety of indigenous plants many of which have not been investigated for their medicinal potential, and only very few have had their extracts separated into the individual compounds they may contain. But, some information is available for Native American herbal uses (Moerman, 2003).

Nine SW/USA species (which were received from Richard Spjut) were included in our Natural Products GRC poster last year. This year, we are reporting on additional species, on residues coming from different extraction solvents, on fractions obtained from counter-current chromatography (CCC), which was performed by Dr. Brent Friesen, and on medicinal potential.

Methods

Dry samples of plant materials were ground into powder and were extracted in a Soxhlex apparatus with hexane or dichloromethane for 4-8 hours. The solutions were then concentrated using a rotavapor, under reduced pressure at about 50-60° C, until no more solvent would come off, giving crude residues which were tested for biological activity. The extracted solid samples were further extracted using methanol at room temperature. These solutions were subjected to the same rotavapor technique (above), producing additional crude residues which were also tested. With the three different solvents, the number of crude residues could be (potentially) as many as three times the number of species.

For the residues which are described (above) as crude, no purification was done on any of them, before the biological testing. Of course the strategy was to try to figure out which ones (if any) might be worthy of separation into individual compounds, which could then be tested.

Thus, CCC was run on two of the crude residues, and selected fractions were submitted for biological testing.

ANTI-TB DATA

Sample	MABA MIC [ug/ml]	LORA MIC [ug/ml]
DA-1*	45.8	41.9
DA-19 (fraction 65 of CCC of DA-1)	47.0	
DA-17*	>50	
DA-3 (fraction 19 of CCC of DA-17)	48.1	
DA-12*	22.7	

*Crude extracts.

Standards	MIC [uM]	MIC [uM]
RMP	0.04	0.76
INH	0.45	> 128
PA824	0.91	1.57

MABA (Microplate Alamar Blue Assay)
LORA (Low Oxygen Recovery Assay)

ANTIBACTERIAL DATA

MICs in μg/mL

Sample	S. aureus	P. aeruginosa	E. faecium
60-1	64	256	64
62-4	32	128	16
64-3	32	128	16

Results and Conclusions

We sent crude residues and selected CCC fractions to the Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, where they were tested for anti-tuberculosis activity, as shown in the "ANTI-TB DATA" table. Using one of our active crude samples, DA-1, as an example, although its MIC (minimum inhibitory concentration) values were appreciably above those of known anti-TB compounds (RMP, INH, and PA824) used as "standards" for comparisons, we submitted DA-1 to CCC, and had selected fractions tested for anti-TB activity, to see if one or more of the fractions had about the same or higher activity than the crude residue. Since DA-19 had about the same activity, we expect that it contains at least one of the active compounds. Therefore, we figure that DA-19 is worthy of further purification.

We did the same thing with DA-17, with DA-3 showing higher activity than DA-17. How much higher we don't know because (of course) we don't know how high >50 really was. DA-3 is also worthy of further purification. Next, we plan to do the same thing with DA-12.

We also sent crude residues to Notre Dame University for antibacterial testing, as shown in the ANTIBACTERIAL DATA table. Three samples had activity against one or more of the bacterial species included in this study. All samples had values of 256 or >256 for E. coli, E. aerogenes, A. baumanii, and K. pneumoniae, so these species are not included in the table.

References

Moerman , D. (2003). Unpublished raw data, University of Michigan-Dearborn, Dearborn, Michigan. Retrieved from http://herb.umd.umich.edu/

Xiao-Tian Liang, Wei-Shuo Fang (editors), Medicinal Chemistry of Bioactive Natural Products (2006), Wiley-Interscience.