10-13-2015

Anti-TB and Antibacterial Activities of Natural Products Extracts

Douglas Armstrong
Olivet Nazarene University, darmstrg@olivet.edu

Nathan Krause
Olivet Nazarene University

Drew Frey
Olivet Nazarene University

Anti-TB and Antibacterial Activities of Natural Products Extracts

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

14th International Conference on the Chemistry of Antibiotics and other Bioactive Compounds
October 13-16, 2015

Abstract

Samples of numerous plant species were received from the southwestern part of the USA from Richard Spjut, and plant samples were collected here in Illinois. All were extracted with typical solvents, giving crude residues, some of which were submitted for anti-TB and/or antibacterial testing. In some cases, crude extracts were subjected to chromatographic methods. Some of the fractions were submitted for testing.

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).

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 ^{o} 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.

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, counter-current chromatography (CCC) was run on two of the crude sw/usa residues by Dr. Brent Friesen, and one crude Illinois residue was subjected to open flash column chromatography (FCC) on silica gel, run by two of the authors (DA and DF), under the direction of Jordan Gunn, doctoral candidate at University of Illinois at Chicago. Selected fractions (from both methods, CCC and FCC) 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	
P8	43.1	

*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, Conclusions, and Further Work

We sent crude residues, selected CCC fractions, and one FCC fraction (P8) 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.

As for CCC, which we applied only to the sw/usa samples, and 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 (a crude residue) 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 at least one of those fractions, 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 another crude residue, DA-17. One of its fractions, DA-3, showed higher activity than DA-17. How much higher we don't know because (of course) we don't know how high ">50" really was (for DA-17) and hence we don't know how low its activity really was. DA-3 is also worthy of further purification. Next, we plan to do the same thing with crude residue, DA-12.

As for FCC, which we applied only to the plant samples collected here in Illinois, fraction P8 had anti-TB activity (43.1), but none of the other FCC fractions had any anti-TB activity. We figure that P8 is worthy of further purification

We also sent crude sw/usa residues to Notre Dame University for antibacterial testing. Three had activity against one or more of the bacterial species included in this study, as shown in the ANTIBACTERIAL DATA table. All samples had values of 256 or >256 for E. coli, E. aerogenes, A. baumanii, and K. pneumoniae, so these bacterial 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.