Supplementary Information for Rationally Minimizing Natural Product Libraries Using Mass Spectrometry

This pdf includes:

In excel workbook:

Supplemental Data Sheet 3: Genera origins of full and rational libraries: Summary of each genera count/percentages in the full libraries, and the count/percentages in the rational libraries.

Supplemental Data Sheet 4: Fungal metadata.

Supplemental Tables

Table S1: Summary of rational library sizes with different parameters for scaffold building. Both positive and negative mode data were collected. Applying our method to the negative mode data, and to low resolution-mimicking data (based on data processing parameters) resulted in similar reduction effectiveness with regards to library size reduction.

	Number of Extracts to 80% Maximum Scaffold diversity	Number of Extracts to 95% Maximum Scaffold diversity	Number of Extracts to 100% Maximum Scaffold diversity
Positive Mode Fungal Data	50 (96.5% reduction)	116 (91.9% reduction)	216 (85.0% reduction)
Negative Mode Fungal Data	41 (97.1% reduction)	96 (93.3% reduction)	174 (87.9% reduction)
Positive Mode Fungal Data: Low Resolution-Mimicking Data	44 (96.9% reduction)	106 (92.6% reduction)	195 (86.4% reduction)
Note: the original library had 1439 fungal extracts.			

Table S2: Hit rates for random sampling of positive mode fungal data. To prove our method's increased hit rates are not an artifact of a reduced absolute library size, we created 1000 iterations of random sampling that were the same size as our rationally reduced library (**Table S1**). Hit rates against each activity assay were then calculated. The lower quartiles, medians, and upper quartiles for all assay hit rates are listed. In every case, our rational method (**Table 1**) outperforms the random sampling.

Activity assay	Lower quartile, median, and upper quartile hit rates for 50 random extracts (1000 iterations)	Lower quartile, median, and upper quartile hit rates for 116 random extracts (1000 iterations)	Lower quartile, median, and upper quartile hit rates for 216 random extracts (1000 iterations)
P. falciparum	8.00% 12.00% 14.00%	9.48% 11.21% 12.93%	9.72% 11.11% 12.50%
T. vaginalis	4.00% 8.00% 10.00%	6.02% 7.76% 9.04%	6.48% 7.87% 8.80%
Neuraminidase	0.00% 2.00% 2.00%	0.86% 1.72% 2.58%	1.39% 1.85% 2.31%

Table S3: Summary of rational library sizes using publicly available data. Data was obtained from reference [22]**,** as deposited in the MassIVE repository under accession number MSV000087728. This collection consisted of a broad taxonomy of pre-fractionated plant extracts.

	Number of Extracts to 80% Maximum Scaffold diversity	Number of Extracts to 95% Maximum Scaffold diversity	Number of Extracts to 100% Maximum Scaffold diversity
Public Data (1,600 total)	104 (93.5% reduction)	233 (85.4% reduction)	408 (74.5% reduction)

Table S4: Higher hit rates for rational libraries, for negative mode analysis of fungal samples. We assessed whether building scaffolds and rational libraries based on the negative polarity MS/MS data yielded the same results as analysis of data collected in positive mode. We found similar patterns, with an increased hit rate in the rational libraries for each activity assay.

Activity assay	Hit rate in full Library	Hit rate in the 80% scaffold diversity library	Hit rate in the 95% scaffold diversity library	Hit rate in the 100% scaffold diversity library
P. falciparum	11.26%	26.83%	18.75%	13.22%
T. vaginalis	7.64%	12.20%	12.50%	9.77%
Neuraminidase	2.57%	7.32%	4.17%	3.45%

Table S5: Classical molecular networking parameters. Parameters used for the positive polarity fungal data, public data, and negative polarity fungal data ("Analysis Parameters"). For low-resolution mimicking parameters, only positive polarity fungal samples were analyzed. See Data Availability section for GNPS job links.

	Analysis Parameters	Low-Resolution Mimicking Parameters
Basic Options		
Precursor Ion Mass Tolerance	0.02 Da	2 Da
Fragment Ion Mass Tolerance	0.02 Da	0.95 Da
Advanced Network Options		
Minimum Pairs Cosine	0.7	0.7
Network TopK	7	7
Maximum Connected Component Size	70	70
Minimum Matched Fragment Ions	4	4
Minimum Cluster Size	4	4
Maximum shift	500 Da	500 Da
Advanced Filtering Options		
Filter below Standard Deviation	0	0
Filter Precursor Window	filter	filter
Filter peaks in 50 Da Window	filter	filter
Filter Spectra from G6 as Blanks Before Networking	don't filter	don't filter
Minimum Peak Intensity	0	0
Filter Library	filter library	filter library

Table S6: Higher hit rates for rational libraries, using low-resolution mimicking parameters and positive mode analysis of fungal samples. To the same positive polarity fungal extract data, classical molecular networking was performed with parameters recommended for low-resolution LC-MS/MS data (See **Table S5** for parameters). The scaffolds and rational libraries were built using this output. We found similar patterns between the low-resolution mimicking and regular parameters, evident by an increased hit rate in the rational libraries for each activity assay.

Activity assay	Hit rate in full Library	Hit rate in the 80% scaffold diversity library	Hit rate in the 95% scaffold diversity library	Hit rate in the 100% scaffold diversity library
P. falciparum	11.26%	13.64%	12.26%	12.31%
T. vaginalis	7.64%	15.91%	10.38%	9.23%
Neuraminidase	2.57%	4.55%	3.77%	4.62%

Table S7: Q-Exactive plus parameters used for fungal data collection. Summary of parameters used in the Thermo Scientific XCalibur software for the positive and negative fungal extract data collection.

Parameter	Value (positive polarity)	Value (negative polarity)
Default Charge State	1	1
Polarity	Positive	Negative
Runtime	12.5 min	12.5 min
Tune data		
Ion source	HESI	HESI
Capillary temp (°C)	320	320
Spray voltage (|V|)	3800	3000
Sheath gas (Thermo Arbitrary units)	35	35
Max spray current (μA)	100	100
Probe heater temp (°C)	350	350
S-lens Radiofrequency level (Thermo Arbitrary units)	50	50
Auxiliary gas (Thermo Arbitrary units)	10	10
Sweep gas (Thermo Arbitrary units)	0	0
Full MS		
Scan Range	100-1500 m/z	100-1500 m/z
Maximum Injection Time	246 ms	246 ms
Resolution (at m/z 200, full width at half maximum (FWHM))	70,000	70,000
AGC Target	1e6	1e6
dd-MS2		
Isolation Window	1.0 m/z	1.0 m/z
Maximum Injection Time	54 ms	54 ms
(N)CE/stepped(N)CE	20 ,40, 60	20, 40, 60
Resolution (at m/z 200, full width at half maximum (FWHM))	17,500	17,500
AGC Target	2e5	2e5

TopN	5	5
dd Settings		
Intensity threshold	1.5e5	1.5e5
Minimum AGC target	8.00e3	8.00e3
Dynamic exclusion	10 s	10 s
Exclude isotopes	on	on
Peptide match	preferred	preferred
AGC:Automatic Gain Control, N(CE): Normalized Collision Energy, HESI: Heated electrospray ionization		

Table S8: MZmine 2.53 parameters used for feature identification: Summary of parameters used on the MS data files in MZmine 2.53. Positive and negative ionization data were analyzed separately.

MZmine 2.53 parameters		
	Positive Ionization	Negative Ionization
Feature Detection		
MS1 Retention Time (min)	0.2-12.5	0.2-12.5
MS1 Noise level	5E5	5E5
MS2 Retention Time (min)	0.2-12.5	0.2-12.5
MS2 Noise level	1E3	1E3
Chromatogram Builder		
Minimum Group Size	5	5
Group Intensity Threshold	5E5	5E5
Minimum Highest Intensity	1E6	1E6
m/z tolerance (ppm)	10	10
Chromatogram Deconvolution		
Algorithm	Local Minimum Search	Local Minimum Search
Chromatogram Threshold	20%	20%
Search Minimum (min)	0.15min	0.15 min
Minimum Relative height	25%	26%
Minimum Absolute height	1E6	1E6
Minimum ratio	1.15	1.3
Peak Duration (min)	0.05-1 min	0.05-1 min
m/z range for MS2	0.01 Da	0.01Da
RT range for MS2 (min)	0.2	0.2
Deisotoping		
m/z tolerance (ppm)	10	10
RT tolerance (min)	0.2	0.2
Monotonic shape	yes	yes

Maximum Charge	3	3
Representative isotope	Most intense	Most intense
Join Aligner		
m/z tolerance (ppm)	10 ppm	10 ppm
Weight for m/z	1	1
RT tolerance (min)	0.2 min	0.2 min
Weight for RT	1	1
Filtering		
Keep peaks with MS2 scan	yes	yes
Reset peak number ID	yes	yes

Supplemental Figures

Fig. S1: Number of features significantly correlated to bioactivity from each assay, along with known molecule derivatives identified in the analysis. In our bioactivity correlation analysis, we found no overlap between significant features or scaffolds between activity assays. As a proof-of-concept, within each assay, we were able to identify MS2 fragmentation matches to derivatives of small molecules with known antimicrobial activity, leucinostatin and altersetin.

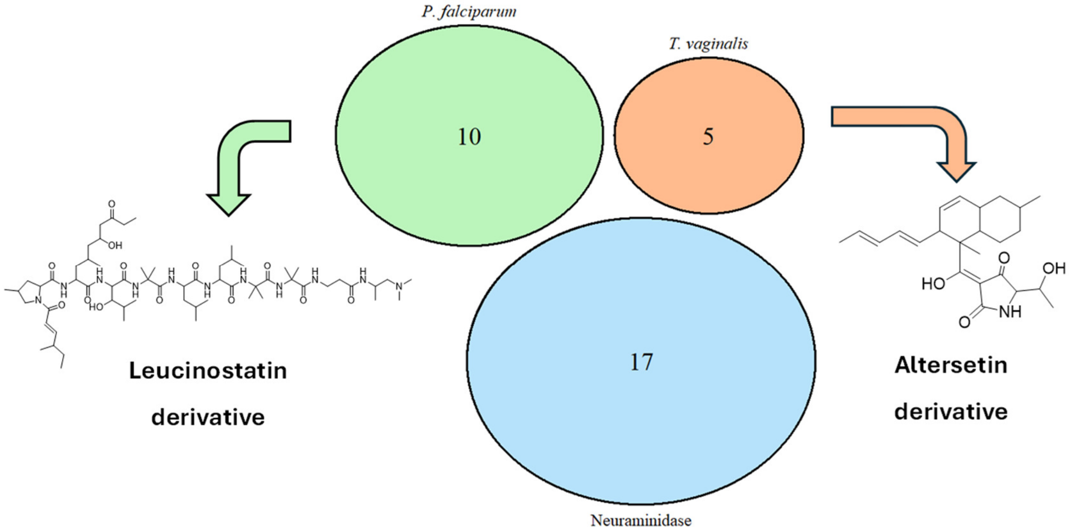


Fig. S2: Annotation overlap between positive and negative ionization modes. Summary of similar annotations found by GNPS molecular networking software.

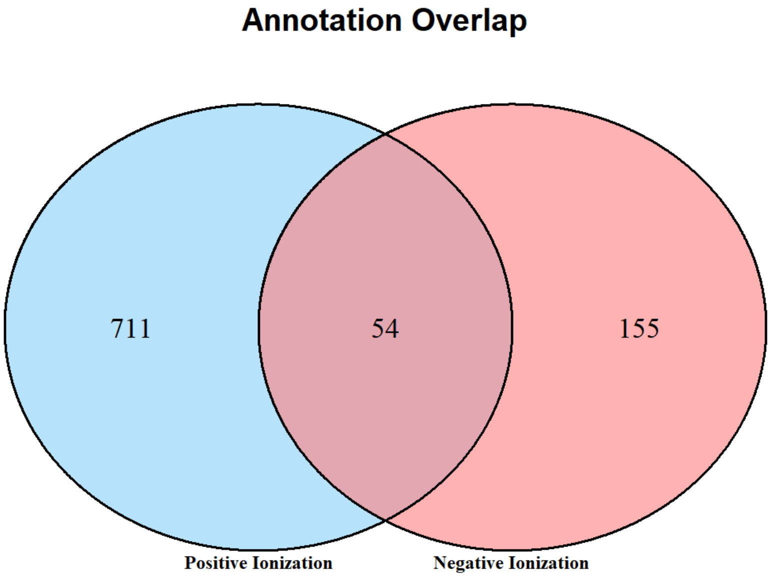


Fig. S3: Rapid accumulation of scaffold diversity with our rational library building method, for negative mode analysis of fungal samples. Scaffold accumulation for random sample iterations (50 iterations, each until 100% scaffold diversity reached) was outperformed by our rational library selection method.

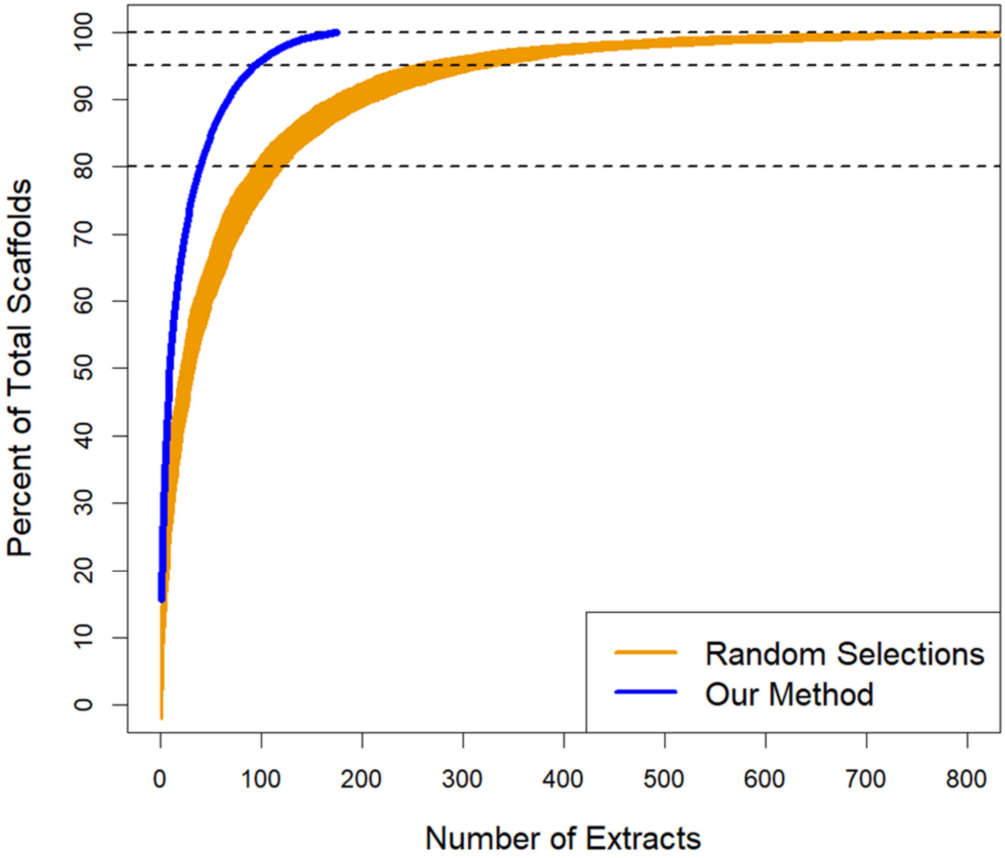


Fig. S4: Rapid accumulation of scaffold diversity with our rational library building method, using low-resolution-mimicking parameters and positive mode analysis of fungal samples. Scaffold accumulation of random sample iterations (50 iterations, each until 100% scaffold diversity reached), compared to our rational library selection method applied on data processed with low resolution-mimicking parameters.

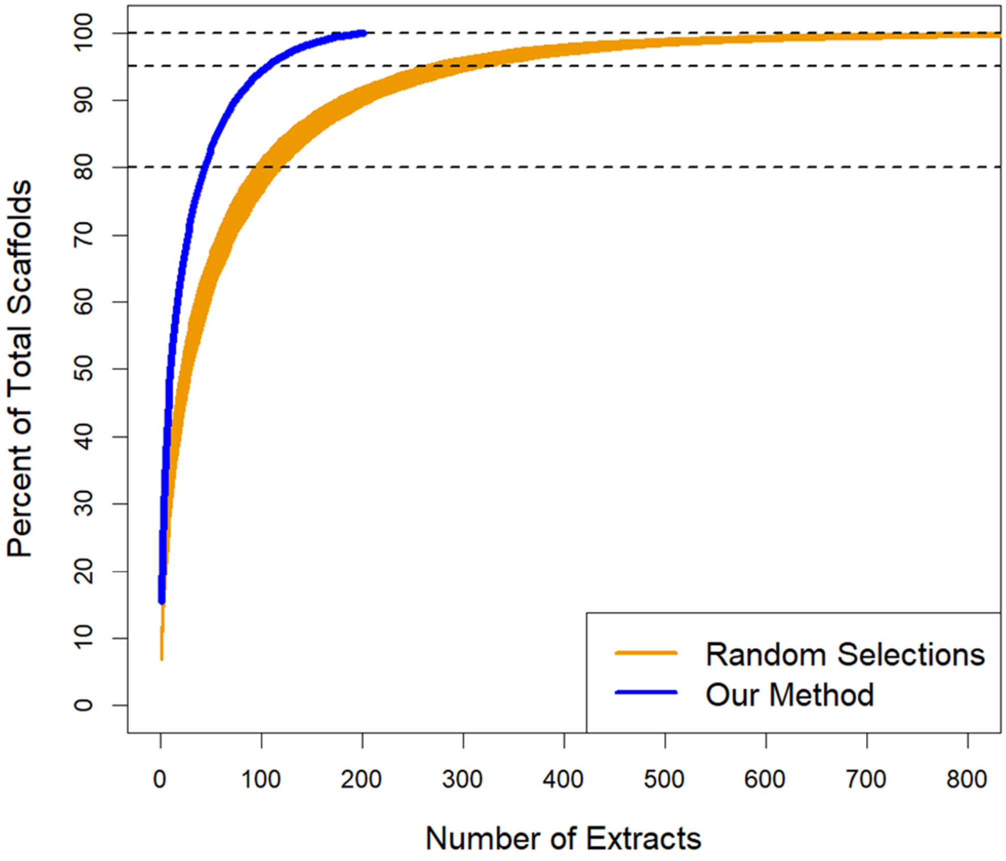


Fig. S5: Comparison of fungal genera presence in bioactive samples versus the full library. Data points above the y = x line indicate genera that are overrepresented in the active extracts relative to their abundance in the full library. Red markers denote statistically significant differences as determined by chi-squared analysis. Only genera with 10 or more extracts in the full library were included in this analysis.

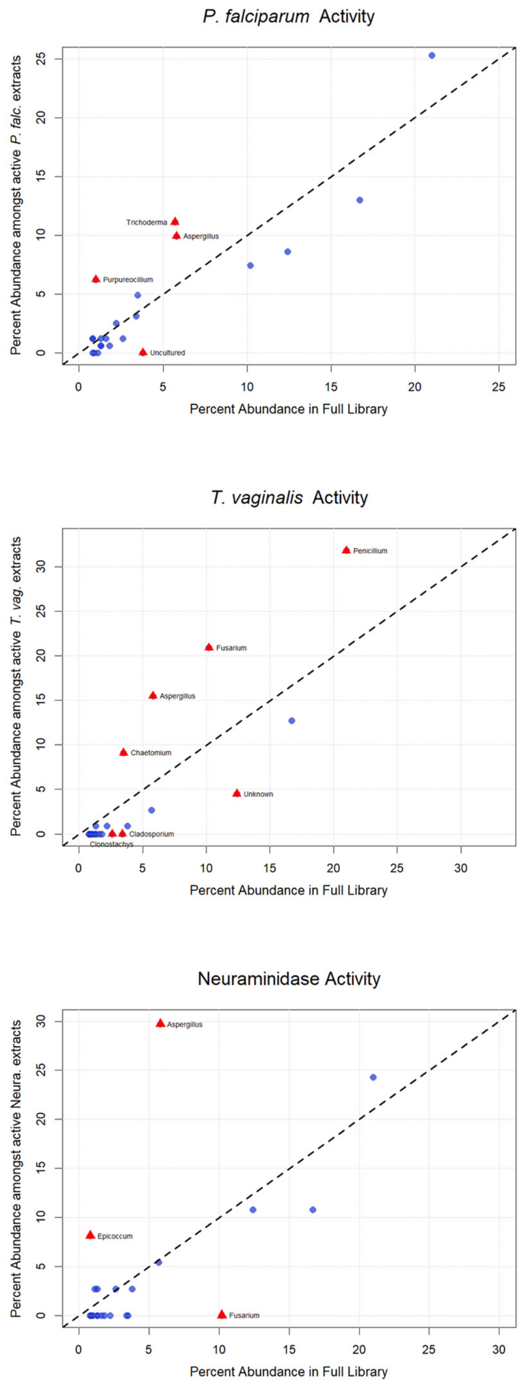


Fig. S6: Venn diagram showing active extracts and their overlap between assays. This shows active crude extracts between the 3 activity assays performed on the full library. Notably, there is minimal overlap between active extracts, other than 50 extracts with both *P. falciparum* and *T. vaginalis* activity, indicating the presence of molecules in these extracts with broad anti-parasitic properties.

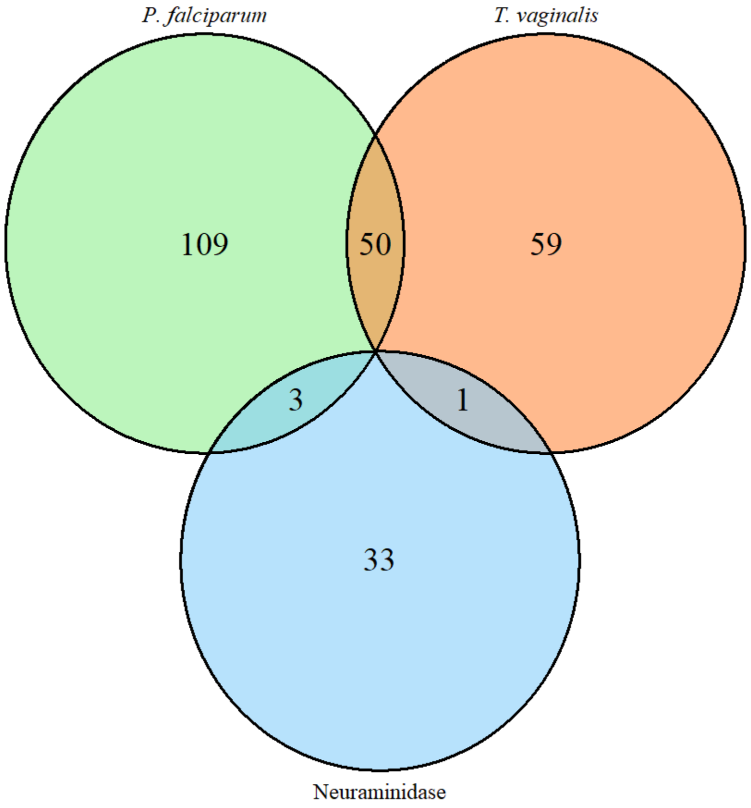


Fig. S7: **Fungal samples collection sites.** Summary of the origins of the fungal extracts used for analysis.

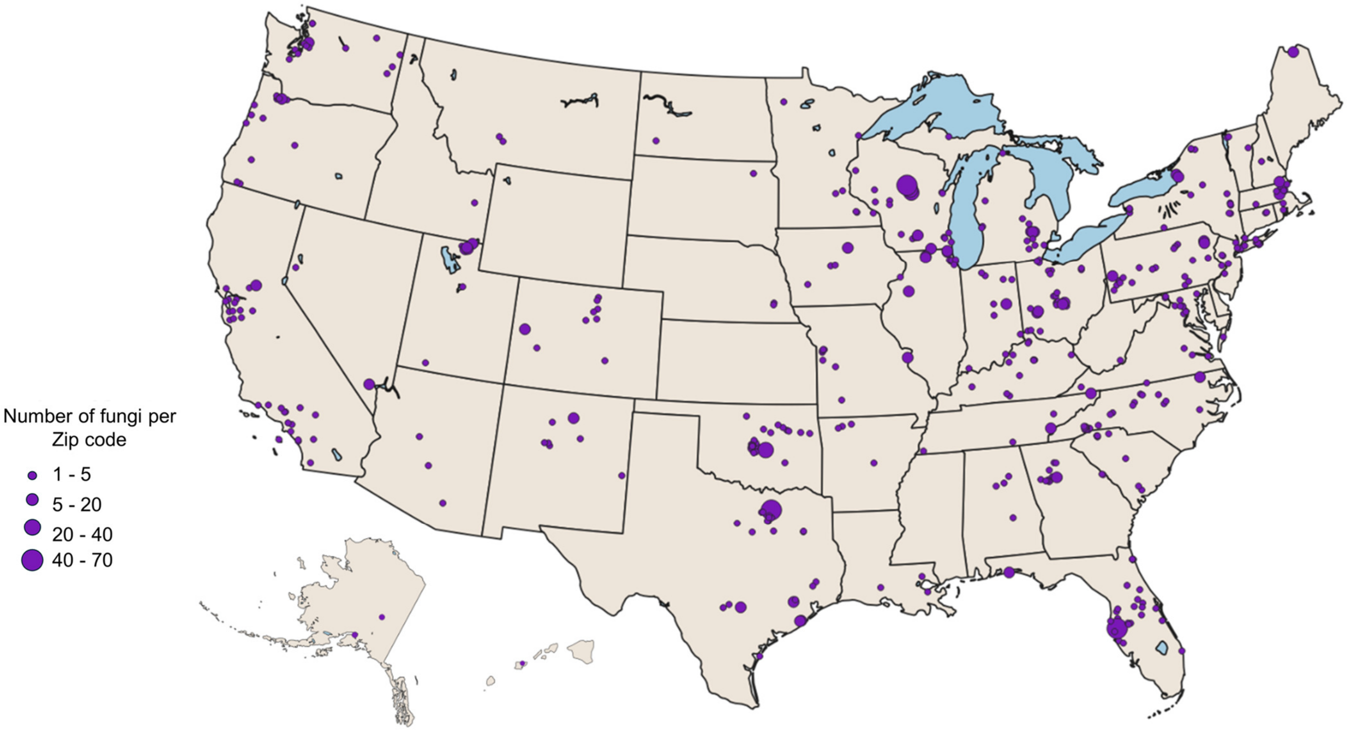


Fig. S8: Fungal samples collection sites, for active extracts. Summary of the origins of the active fungal extracts identified for each of the 3 assays. Green dots represent anti-*P. falciparum* activity, orange represents anti-*T. vaginalis* activity, and blue dots represent anti-neuraminidase activity. The size of the dots correlates to the number of active isolates from that area.

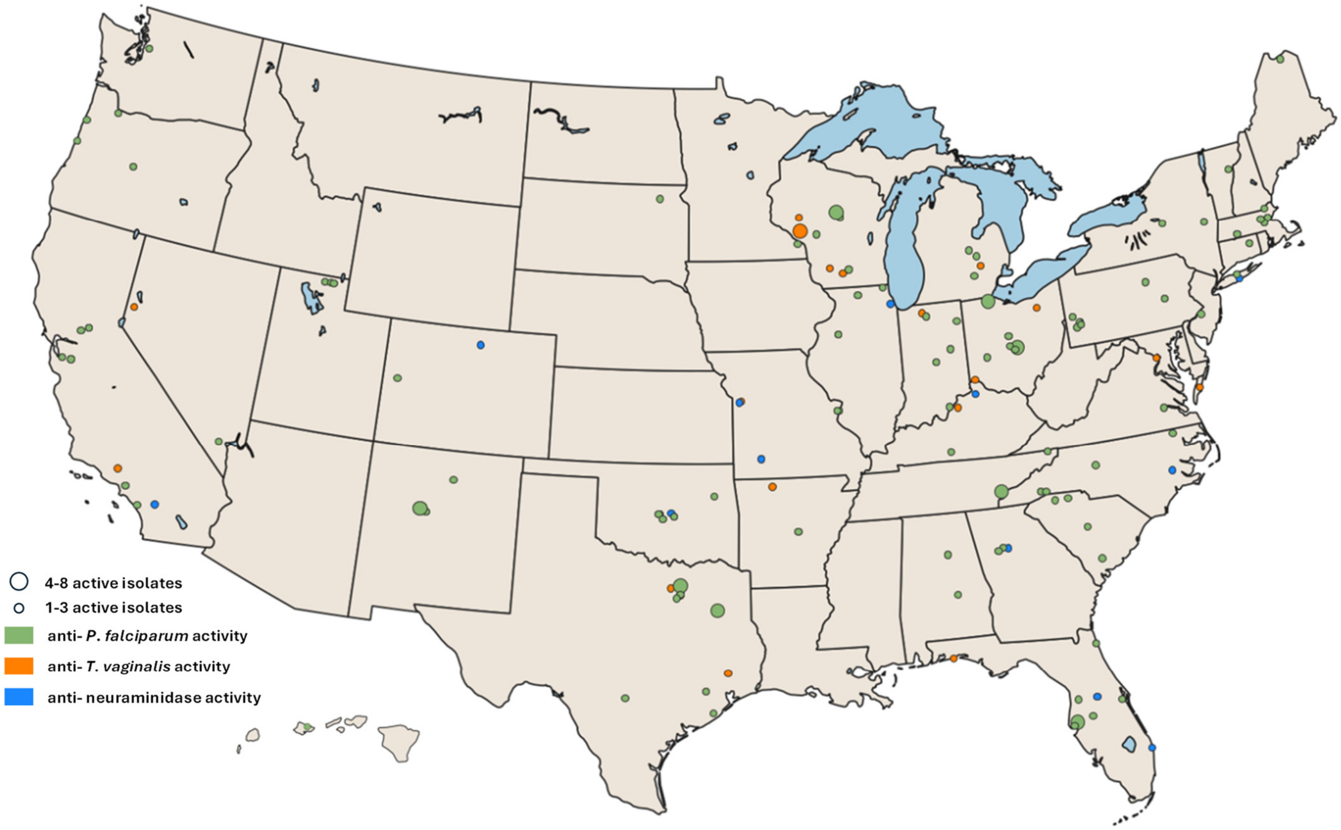
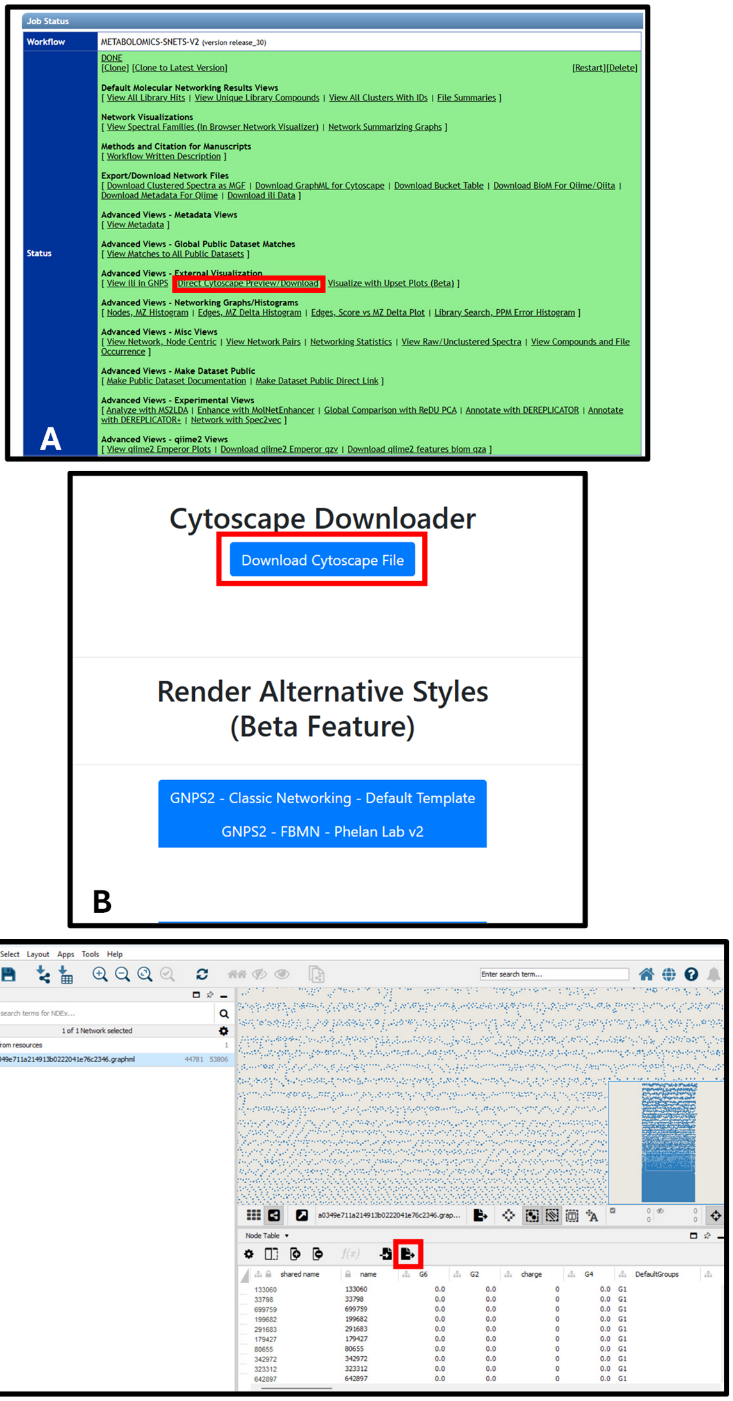

○ 4-8 active isolates
○ 1-3 active isolates
anti- P. falciparum activity
anti- T. vaginalis activity
anti- neuraminidase activity

Fig. S9: Instructions for node table download from the classical molecular networking job page. When the classical molecular networking job is complete, access to a completed job status page appears (A). From there, select “Direct Cytoscape Preview/Download”, which leads to (B). Download the file and open Cytoscape. You will need to have the Cytoscape software installed. The networking job should appear and look similar to (C). Select “Export table to file” and save the file in a desired location as a .csv file.



Job Status

Workflow: METABOLOMICS-SNETS-V2 (version release_30)

Status:

DONE
[Clone] [Clone to Latest Version] [Restart][Delete]

Default Molecular Networking Results Views
[View All Library Hits | View Unique Library Compounds | View All Clusters With IDs | File Summaries]

Network Visualizations
[View Spectral Families (In Browser Network Visualizer) | Network Summarizing Graphs]

Methods and Citation for Manuscripts
[Workflow Written Description]

Export/Download Network Files
[Download Clustered Spectra as MGF | Download GraphML for Cytoscape | Download Bucket Table | Download BioM For Qiime/Qiita | Download Metadata For Qiime | Download ili Data]

Advanced Views - Metadata Views
[View Metadata]

Advanced Views - Global Public Dataset Matches
[View Matches to All Public Datasets]

Advanced Views - External Visualization
[View ili in GNPS | Direct Cytoscape Preview/Download | Visualize with Upset Plots (Beta)]

Advanced Views - Networking Graphs/Histograms
[Nodes, MZ Histogram | Edges, MZ Delta Histogram | Edges, Score vs MZ Delta Plot | Library Search, PPM Error Histogram]

Advanced Views - Misc Views
[View Network, Node Centric | View Network Pairs | Networking Statistics | View Raw/Unclustered Spectra | View Compounds and File Occurrence]

Advanced Views - Make Dataset Public
[Make Public Dataset Documentation | Make Dataset Public Direct Link]

Advanced Views - Experimental Views
[Analyze with MS2LDA | Enhance with MolNetEnhancer | Global Comparison with ReDU PCA | Annotate with DEREPLICATOR | Annotate with DEREPLICATOR+ | Network with Spec2vec]

Advanced Views - qiime2 Views
[View qiime2 Emperor Plots | Download qiime2 Emperor qzv | Download qiime2 features biom qza]

Cytoscape Downloader

Download Cytoscape File

Render Alternative Styles (Beta Feature)

GNPS2 - Classic Networking - Default Template

GNPS2 - FBMN - Phelan Lab v2

Network

Enter search terms for NDEx...

1 of 1 Network selected

Created from resources 1

a0349e711a214913b0222041e76c2346.graphml 44781 53806

a0349e711a214913b0222041e76c2346.grap...

Node Table

shared name	name	G6	G2	charge	G4	DefaultGroups
133060	133060	0.0	0.0	0	0.0	G1
33798	33798	0.0	0.0	0	0.0	G1
699759	699759	0.0	0.0	0	0.0	G1
199682	199682	0.0	0.0	0	0.0	G1
291683	291683	0.0	0.0	0	0.0	G1
179427	179427	0.0	0.0	0	0.0	G1
80655	80655	0.0	0.0	0	0.0	G1
342972	342972	0.0	0.0	0	0.0	G1
323312	323312	0.0	0.0	0	0.0	G1
642897	642897	0.0	0.0	0	0.0	G1

Supplementary Data (excel workbook):

Supplemental Data Sheet 1: Significant bioactive features for each ionization method. Counts of features significantly correlated with bioactivity, categorized by ionization method (positive or negative ion mode). On the left, the table outlines the method used for rational library generation. Rational libraries are created using Classical Molecular Networking (CMN) scaffolds, representing jobs for positive, negative, or low-resolution-mimicking positive ionization. For each CMN job, results from the three bioassays are presented. Within the table, counts of total significant features, and their retention in the rational libraries is provided. Since the same data was used for all correlation calculations, the total number of significant features in the full dataset remains constant for each bioassay, irrespective of the CMN job.

Supplemental Data Sheet 2: Changing classical molecular networking parameters has little impact on library size and hit rates. Summary of hit rates and library sizes with different multiple molecular networking parameters. Note that other than the parameters listed in the table, all other parameters are the same as listed in Table S5.

Supplemental Data Sheet 3: Genera origins of full and rational libraries: Summary of each genera count/percentages in the full libraries, and the count/percentages in the rational libraries. A Chi-squared significance test p-value is also listed to readily identify genera significantly over or underrepresented in the rational libraries

Supplemental Data Sheet 4: Fungal metadata. Summary of each fungal extract, the zip code the sample was collected, the soil extract origin, and the genus.