

**Integrated *In Vitro-In Vivo* Evaluation of Fulvic Acid-Based Formulations:
Cytocompatibility, Genotoxicity, Immunomodulation, and Gut Microbiota
Profiling**

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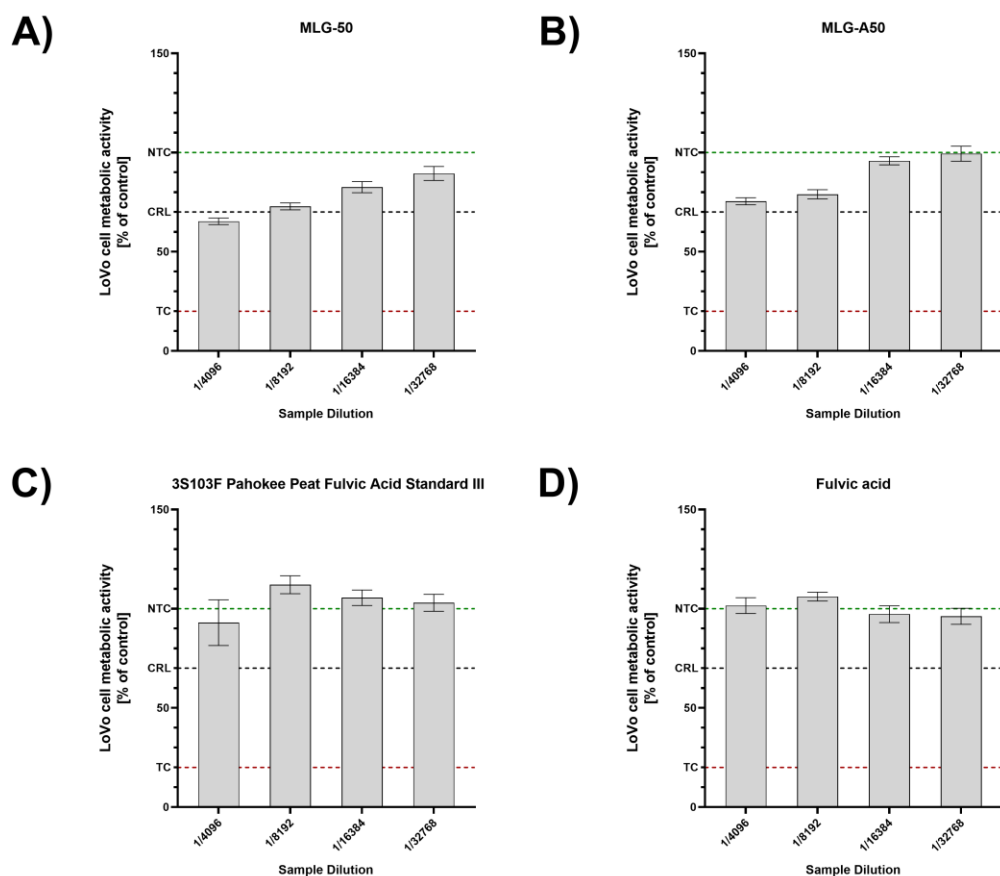
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25 **Table 1.** Compositional comparison of fulvic acid formulations used in this study¹.

Component	MLG-50	MLG-A50
Fulvic acid (%)	3.35%	4.39%
pH	~1.97	~12.10
Calcium (Ca, ppm)	455 ppm	104 ppm
Magnesium (Mg, ppm)	850 ppm	49.7 ppm
Sodium (Na, ppm)	8,000 ppm	4,040 ppm
Specific gravity	1.09	1.04
Total dissolved solids (TDS %)	15.0	10.1

26 ¹Values are taken from ISO-17025 laboratory testing and batch COAs; Al and heavy metals (As,
27 Cd, Pb, Hg) were below safety thresholds and are reported in full in the deposited COAs and
28 available upon request.

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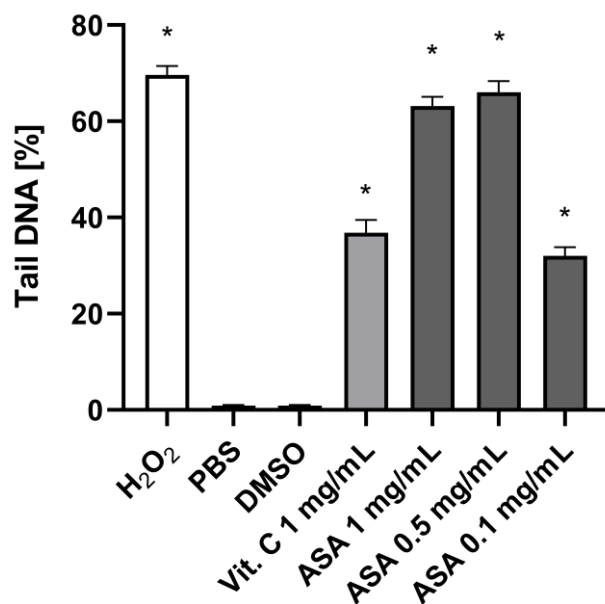


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Figure S1. Effect of fulvic acid-containing preparations on LoVo cell metabolic activity after 72 h exposure. Cells were treated with MLG-50 (**A**), MLG-A50 (**B**), 3S103F Pahokee Peat Fulvic Acid Standard III (**C**), or Fulvic Acid Cayman (**D**) at dilutions of 1/8192, 1/16384, and 1/32768. After 72 hours, metabolic activity was assessed using a colorimetric viability assay. Untreated cells served as the control. Data represent mean $\pm$ SEM from three independent experiments.

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40 **Figure S2.** Genotoxic effect observed on HepG2 cell lines after 24 hrs incubation with control
 41 compounds. Nucleus damage presented as tail DNA [%] (Lucia Comet Assay™, Laboratory
 42 Imaging). PBS was used as a non-treated control (NTC), and 50 µg/mL H₂O₂ as a treated control.
 43 Graphs represent the mean values with the standard error of the mean (SEM). * indicate statistical
 44 significance vs NTC (p<0.05)

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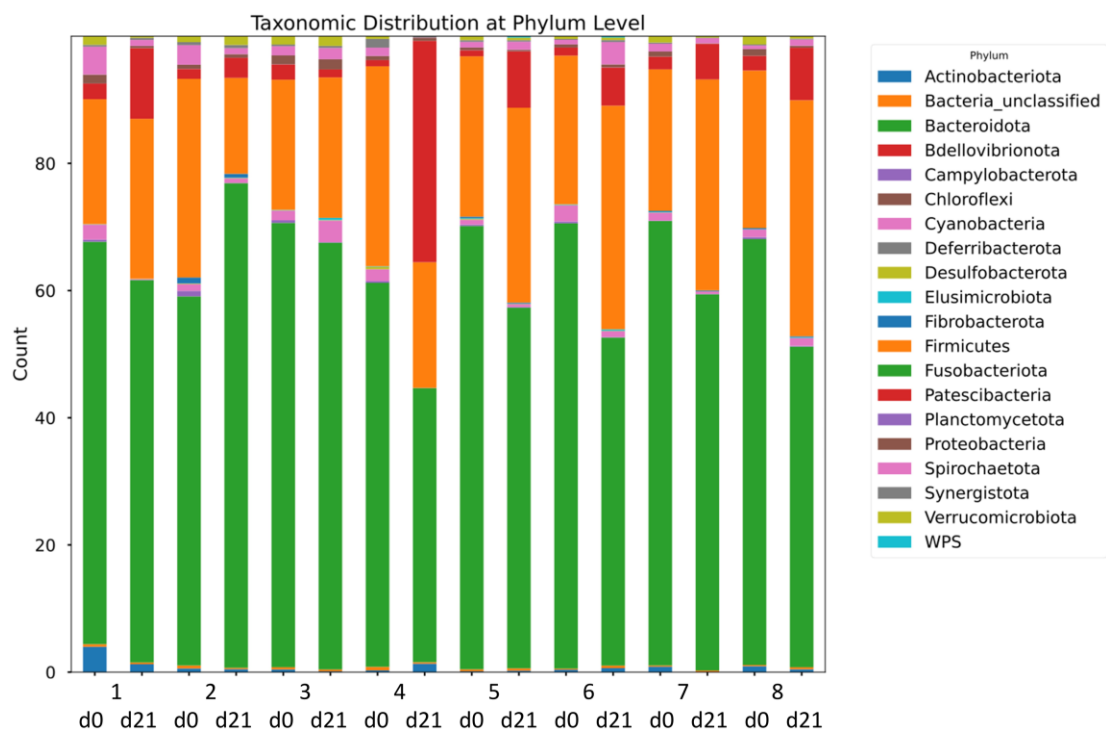


Figure S3. Phylum-level gut microbiota profiles in individual Himalayan guinea pigs before and after fulvic-acid supplementation. Stacked bar plots show the relative abundance (percent of total counts) of bacterial phyla identified by 16S rRNA gene fragment amplicon sequencing and Mothur-based taxonomic classification. Each animal (1–8) is shown at baseline (d0) and post-intervention (d21). Colors denote phyla as indicated in the legend, and phyla present at very low abundance are retained to illustrate inter-individual variability.

Supplementary methods

Bioinformatic pipeline for 16S rDNA analyses

Mothur analyses were applied to sequencing reads using `make.contigs` for assembly, `screen.seqs` for quality filtering, `unique.seqs` for dereplication, and `align.seqs` to align reads against the SILVA reference alignment (v138.1) trimmed to the V3–V4 region. After gap and noise removal (`filter.seqs`, `pre.cluster`), chimeric sequences were excluded using `chimera.vsearch`. Reads were classified with `classify.seqs` using the SILVA taxonomy, and non-bacterial and ambiguous lineages (e.g., chloroplasts, mitochondria, Archaea, and Eukaryota) were removed with `remove.lineage`. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity (`cluster.split`) and quantified per sample (`make.shared`), with taxonomy assigned via `classify.otu`. Microbial community richness and diversity for the second round of experiment were evaluated using alpha-diversity metrics, including the Shannon index, while beta-diversity and longitudinal comparisons (day 0 vs. day 21) enabled assessment of microbiota shifts induced by fulvic acid treatments. All processing steps were performed identically across groups to ensure comparability between conditions and experimental phases.