

Mexican Oregano (*Lippia berlandieri* Schauer and *Poliomintha longiflora* Gray) Essential Oils Induce Cell Death by Apoptosis in *Leishmania (Leishmania) mexicana* Promastigotes

Karla Fabiola Chacón-Vargas¹, Luvia Enid Sánchez-Torres^{2,*}, Mónica L. Chávez-González³, Jaime R. Adame-Gallegos¹ and Guadalupe Virginia Nevárez-Moorillón^{1,*}

Supplementary files

Table S1. Chemical compounds identified in the essential oils of *Lippia berlandieri* and *Poliomontha longiflora*

Compounds	%
<i>Lippia berlandieri</i> EO	
Alpha-tujhone	0.049
β -cis-Ocimene	0.09
Anisole	0.259
2,5-diethyl-3,6-dimethylpyrazine	0.64
Thymol	7.863
Carvacrol	33.781
M-cymen-4-ol	0.108
Phenol-2-methoxy (Guaiacol)	0.305
Adamantane,2-methyl	0.273
Gitoxigenin	0.941
Alpha-eudesmol	0.123
<i>Poliomintha longiflora</i> EO	
β -pinene	0.637
Durene	21.064
Thymol	23.46
Carvacrol	18.35

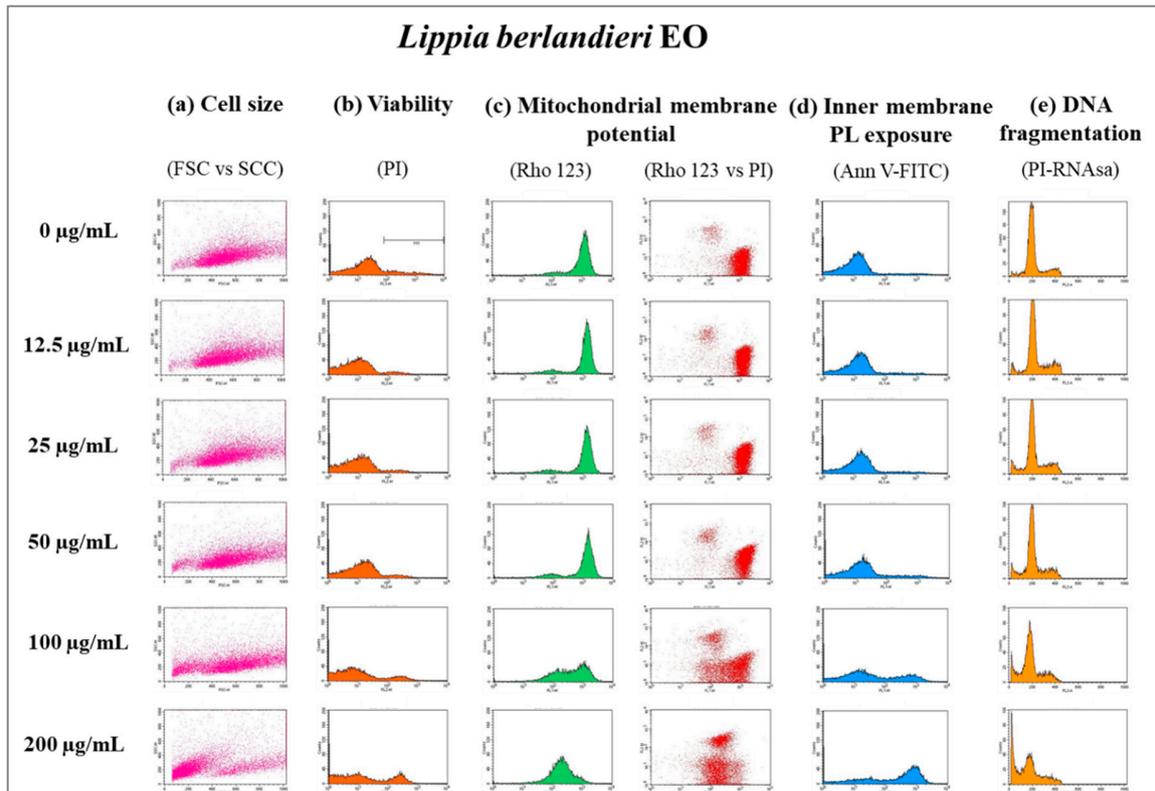


Figure S1. Characterization of the leishmanicidal activity of *Lippia berlandieri* EO by flow cytometry. *Leishmania mexicana* promastigotes were treated with 0, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$ of *L. berlandieri* EO, incubated for 24 h and different markers of cell death were analyzed by flow cytometry. (a) Changes in size (FSC vs SSC); (b) Plasma membrane integrity; (c) Mitochondrial membrane potential (monoparametric and biparametric with PI analysis); (d) Inner membrane phospholipids (PL) exposure; (e) DNA fragmentation. The experiment was performed in triplicate, and representative images are shown.

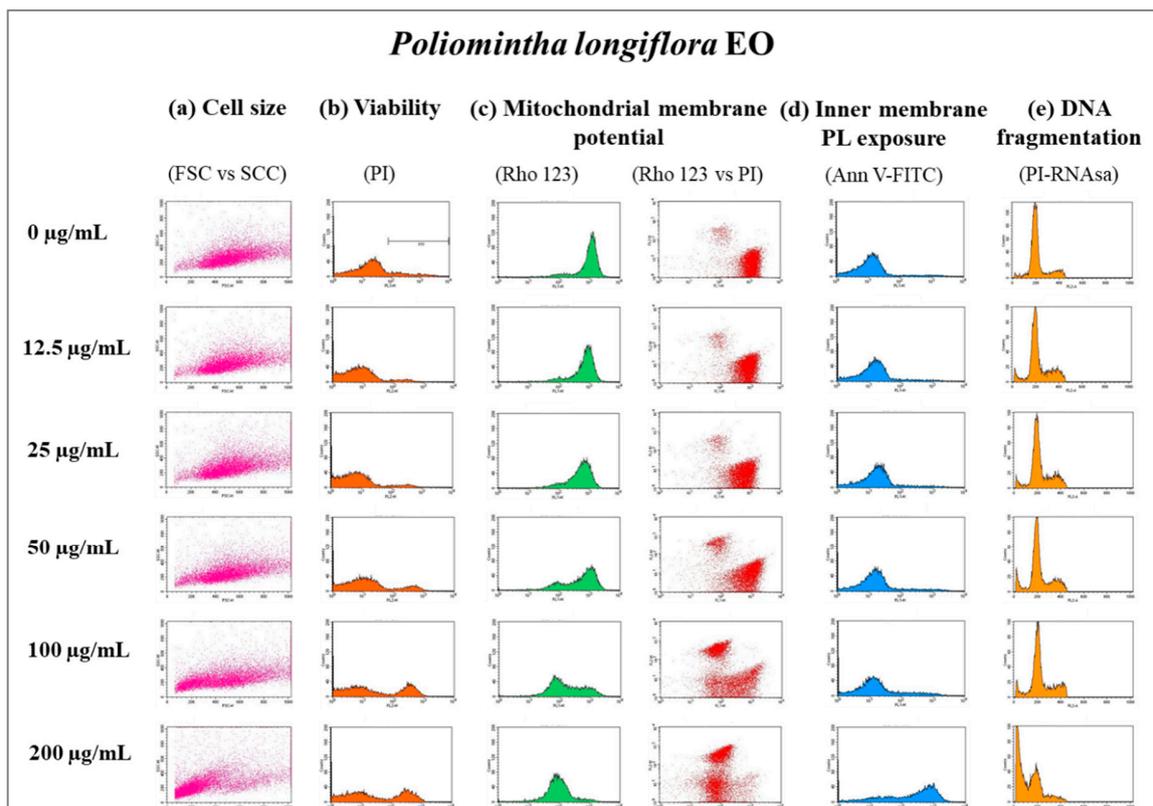


Figure S2. Characterization of the leishmanicidal activity of *Poliomintha longiflora* EO by flow cytometry. *Leishmania mexicana* promastigotes were treated with 0, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$ of *P. longiflora* EO, incubated for 24 h and different markers of cell death were analyzed by flow cytometry. (a) Changes in size (FSC vs SSC); (b) Plasma membrane integrity; (c) Mitochondrial membrane potential (monoparametric and biparametric with PI analysis); (d) Inner membrane phospholipids (PL) exposure; (e) DNA fragmentation. The experiment was performed in triplicate, and representative images are shown.

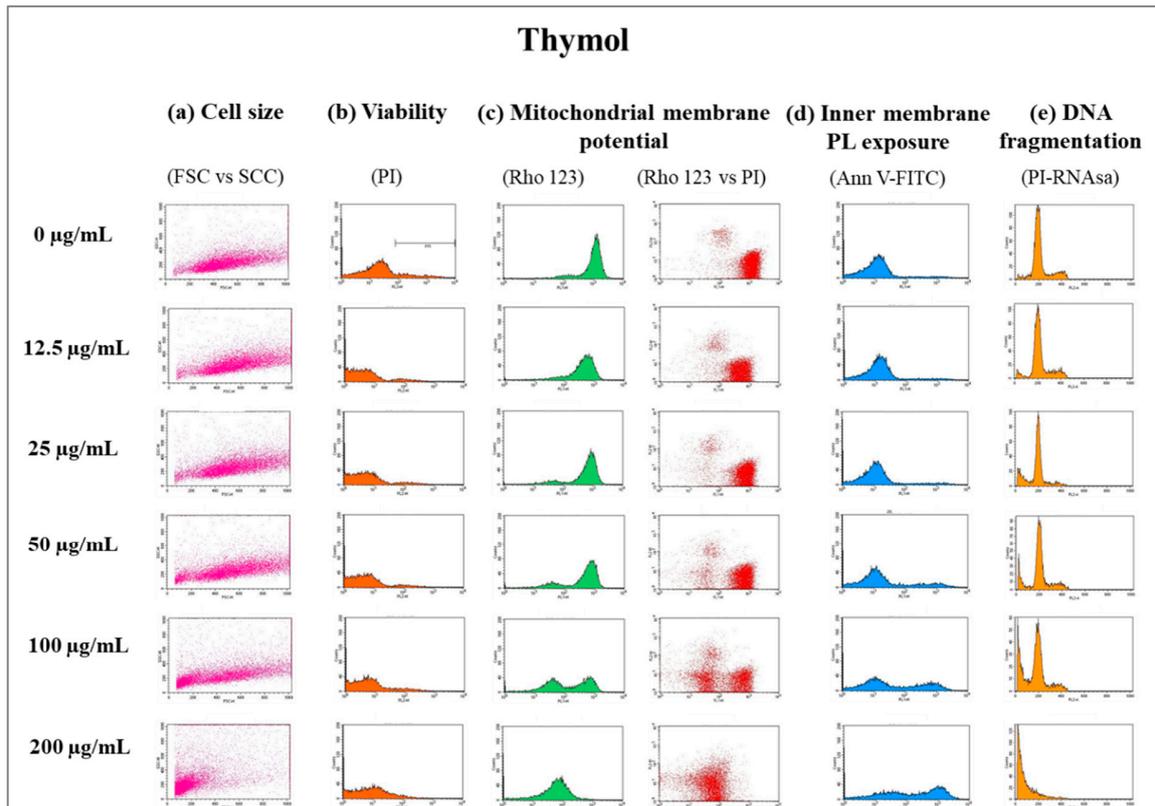


Figure S3. Characterization of the leishmanicidal activity of thymol by flow cytometry. *Leishmania mexicana* promastigotes were treated with 0, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$ of thymol incubated for 24 h and different markers of cell death were analyzed by flow cytometry. a) Changes in size (FSC vs SSC); b) Plasma membrane integrity; d) Mitochondrial membrane potential (monoparametric and biparametric with PI analysis); c) Inner membrane phospholipids (PL) exposure; (e) DNA fragmentation. The experiment was performed in triplicate, and representative images are shown.

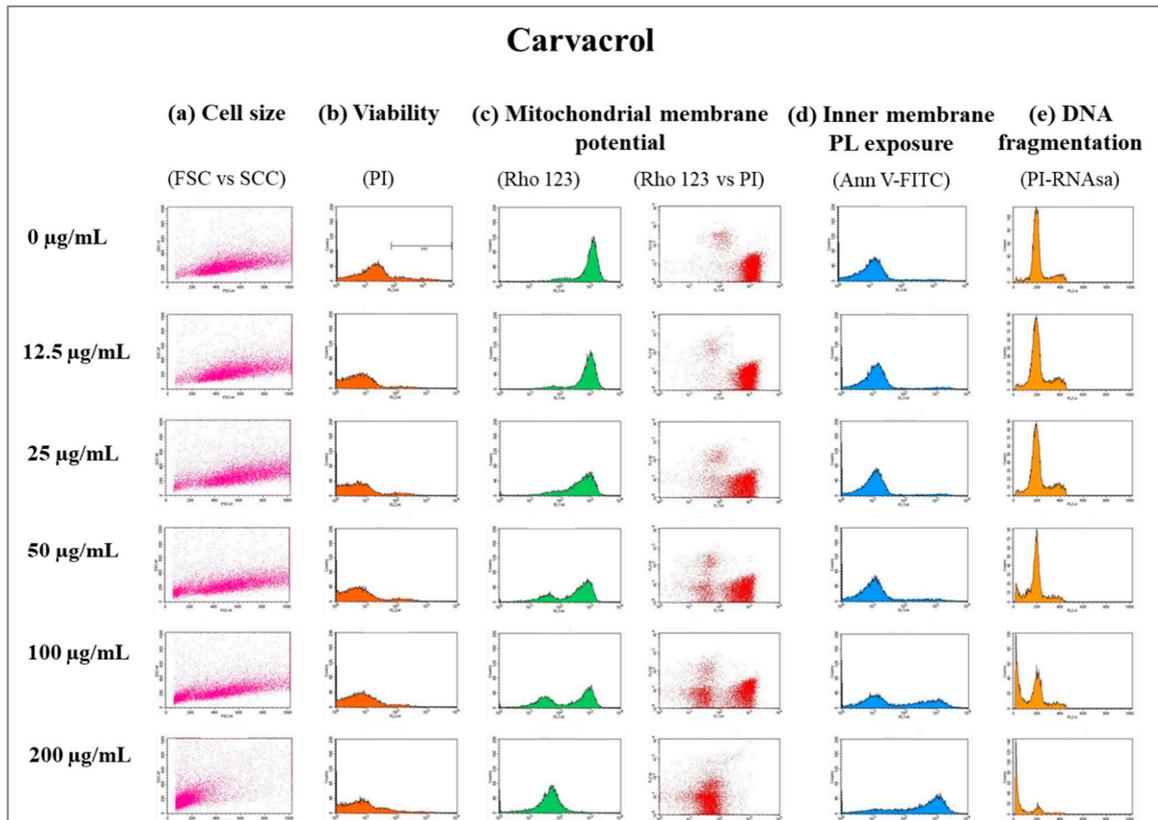


Figure S4. Characterization of the leishmanicidal activity of carvacrol by flow cytometry. *Leishmania mexicana* promastigotes were treated with 0, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$ of carvacrol, incubated for 24 h and different markers of cell death were analyzed by flow cytometry. a) Changes in size (FSC vs SSC); b) Plasma membrane integrity; d) Mitochondrial membrane potential (monoparametric and biparametric with PI analysis); c) Inner membrane phospholipids (PL) exposure; (e) DNA fragmentation. The experiment was performed in triplicate, and representative images are shown.