The enhanced activity of a plant mixture from the Brazilian Caatinga biome against venereal trichomonads confirms the traditional use

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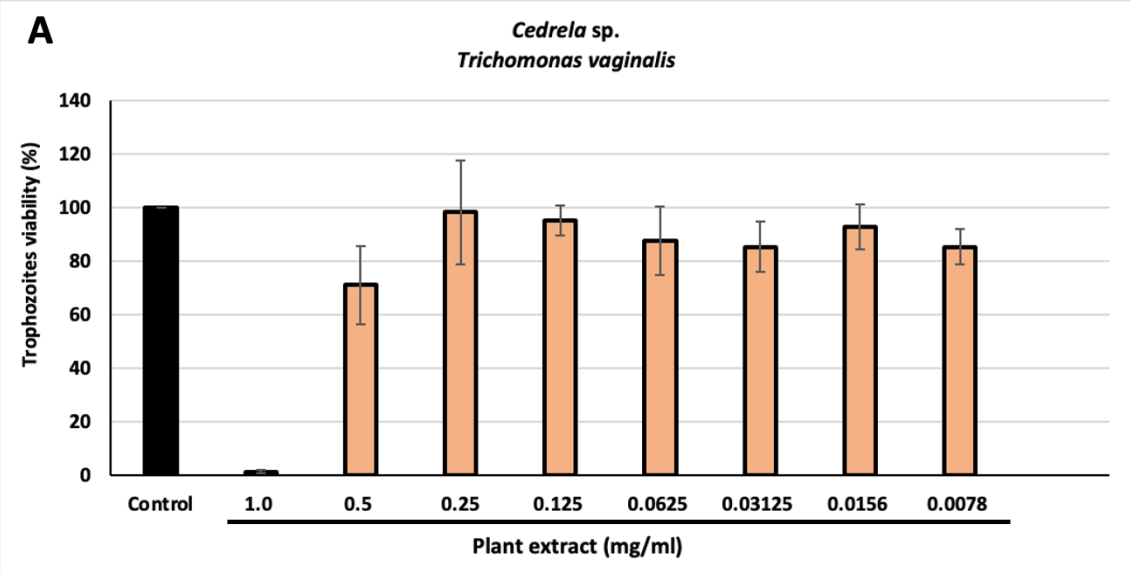
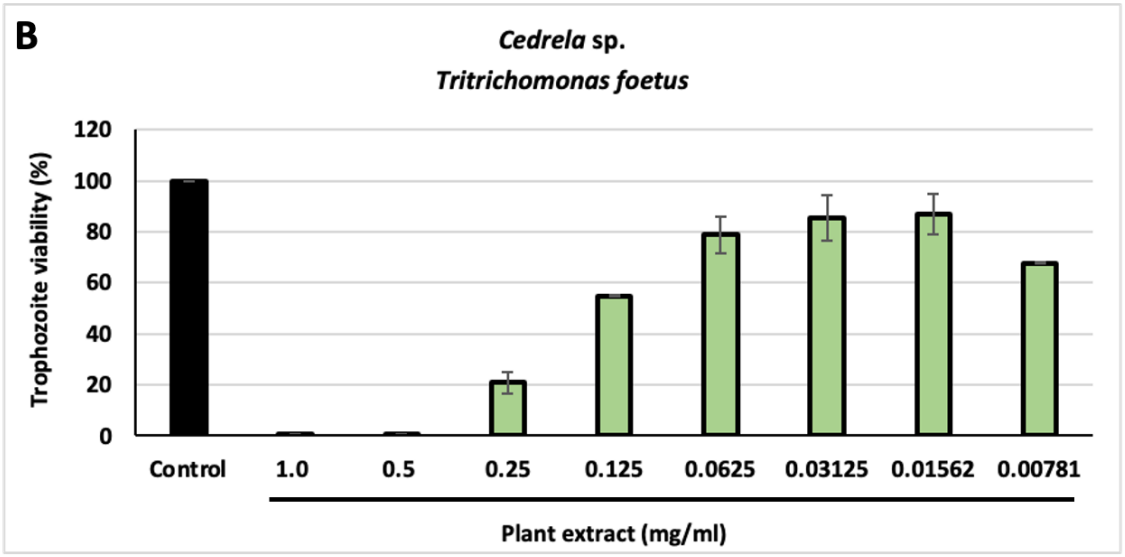
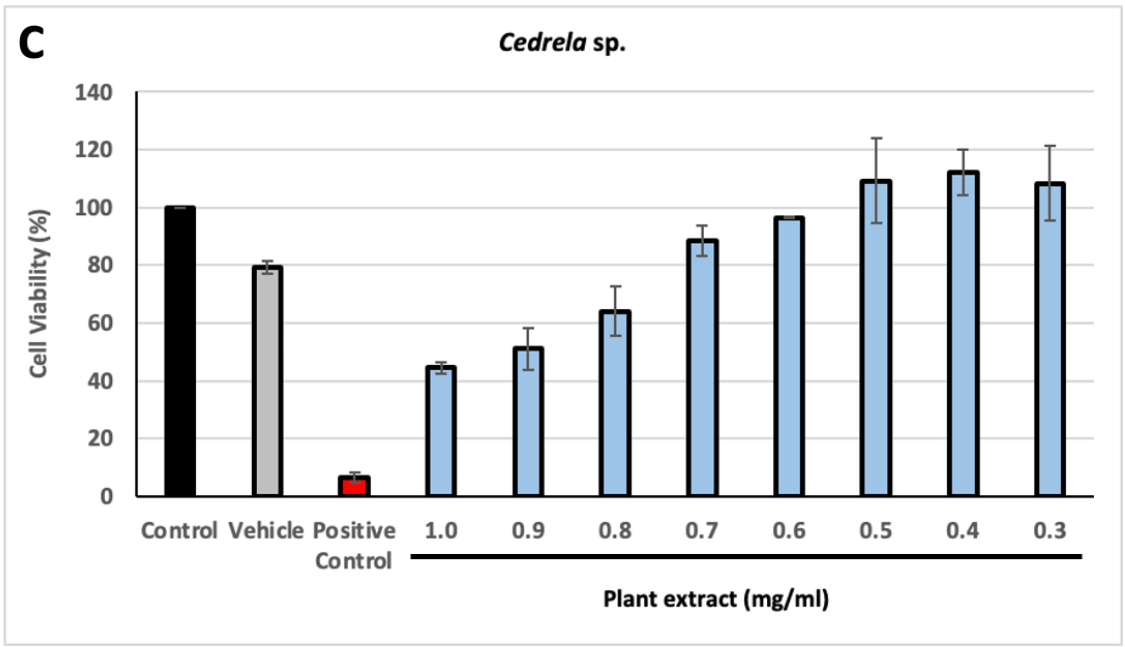
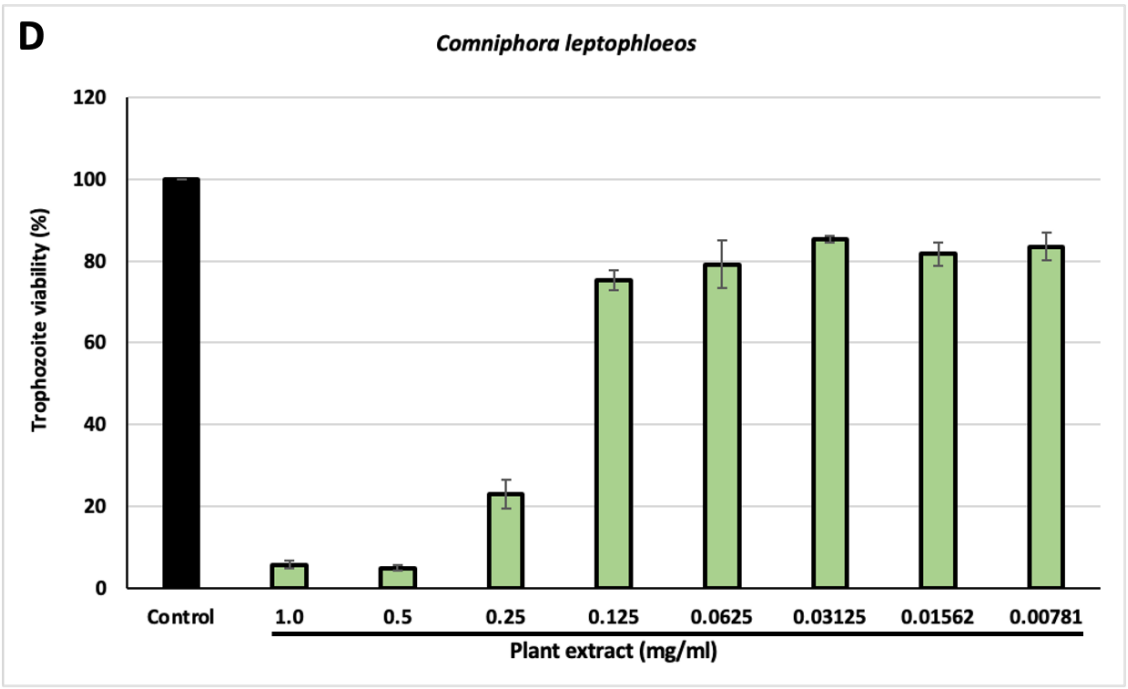
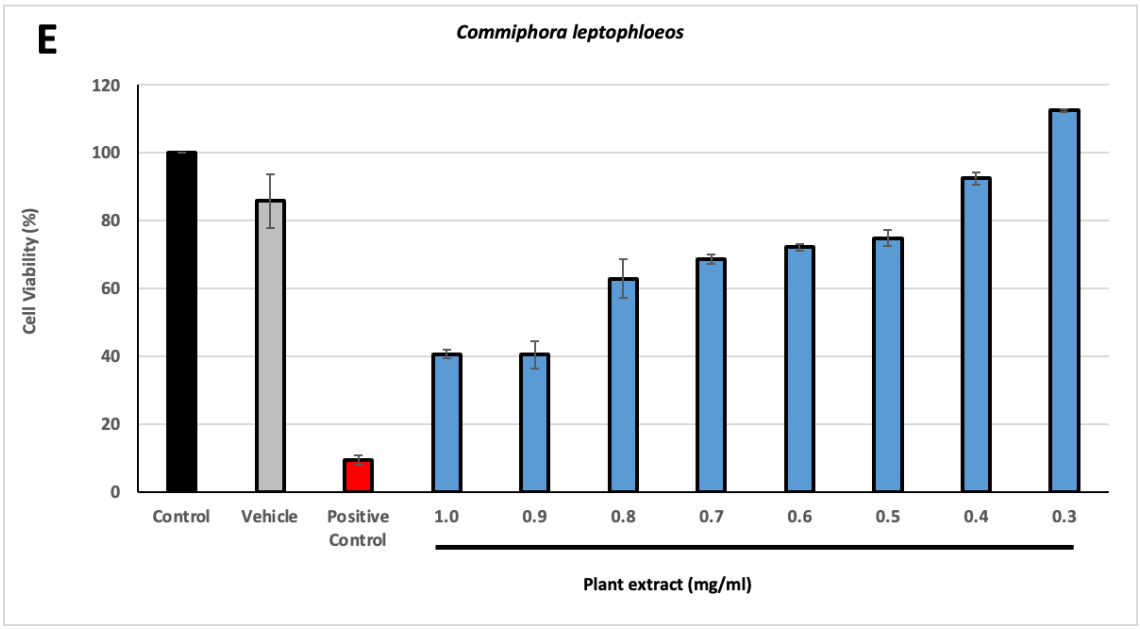
Supplementary Material

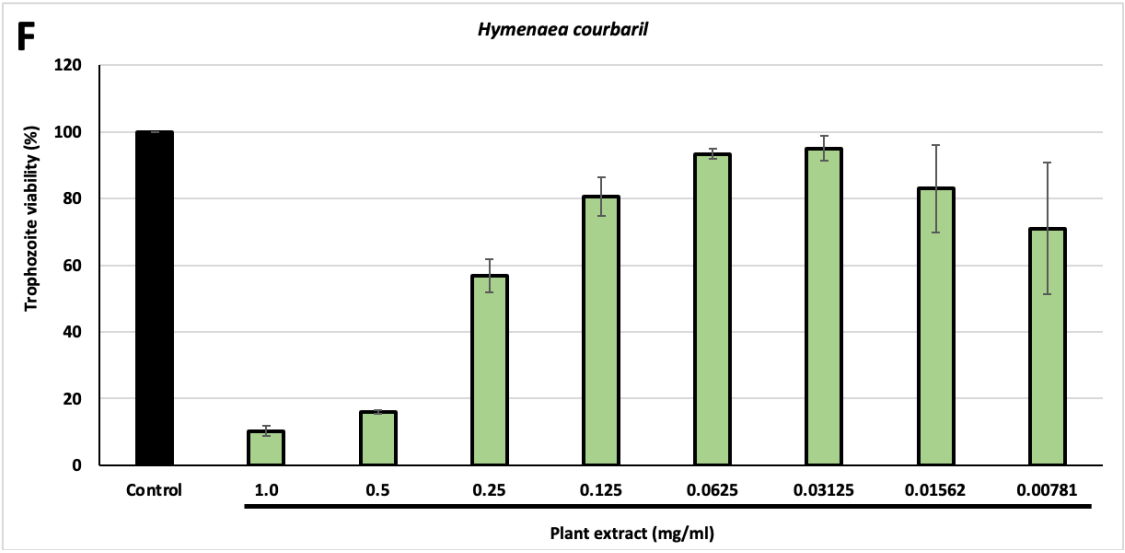


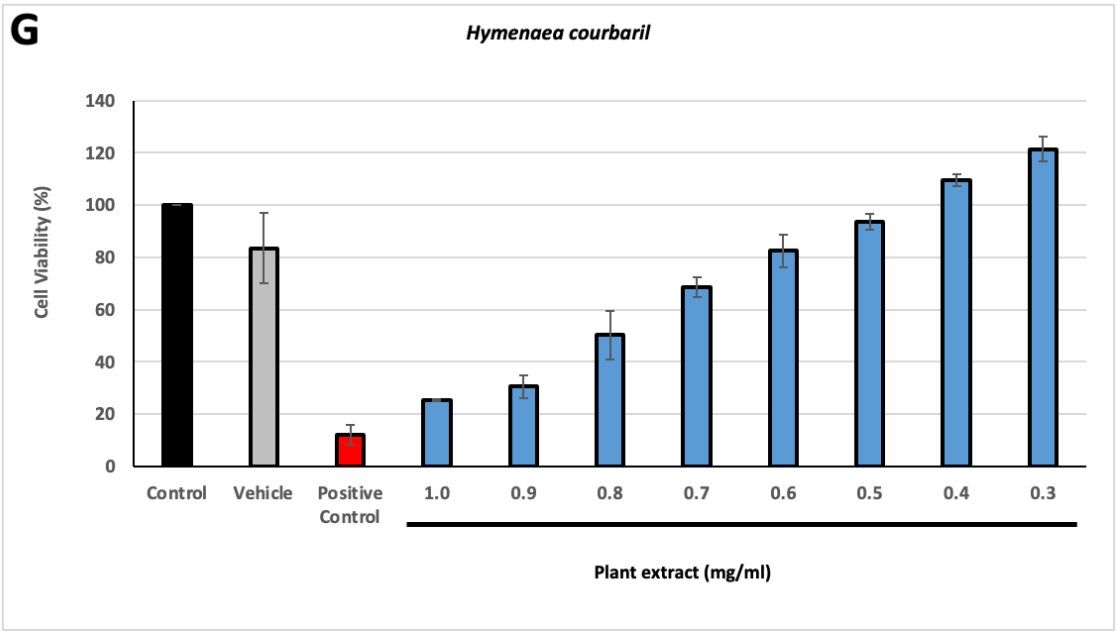

B
Cedrela sp.
Tritrichomonas foetus
Trophozoite viability (%)
120
100
80
60
40
20
0
Control
1.0
0.5
0.25
0.125
0.0625
0.03125
0.01562
0.00781
Plant extract (mg/ml)

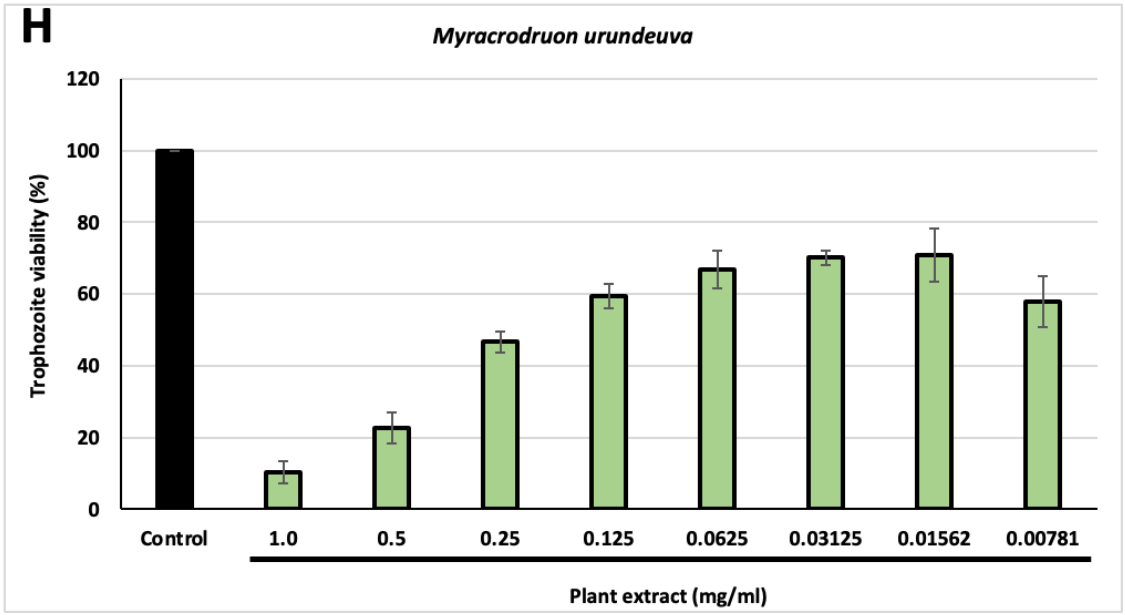

C
Cedrela sp.
Cell Viability (%)
140
120
100
80
60
40
20
0
Control
Vehicle
Positive Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Plant extract (mg/ml)

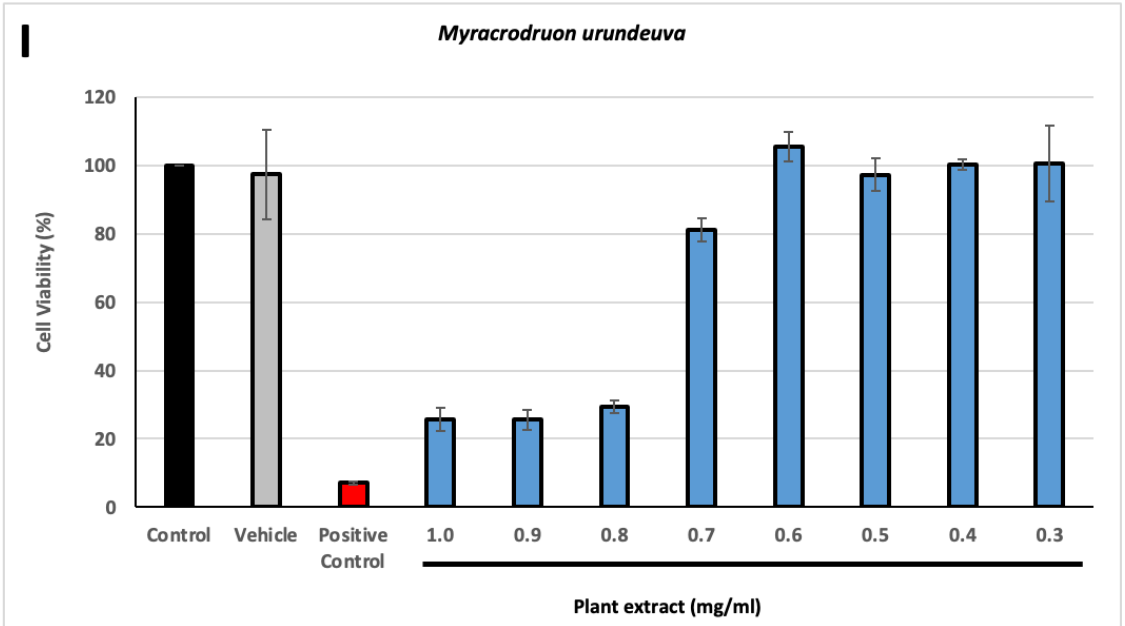

D
Comniphora leptophloeos
Trophozoite viability (%)
120
100
80
60
40
20
0
Control
1.0
0.5
0.25
0.125
0.0625
0.03125
0.01562
0.00781
Plant extract (mg/ml)


E
Commiphora leptophloeos
Cell Viability (%)
120
100
80
60
40
20
0
Control
Vehicle
Positive Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Plant extract (mg/ml)

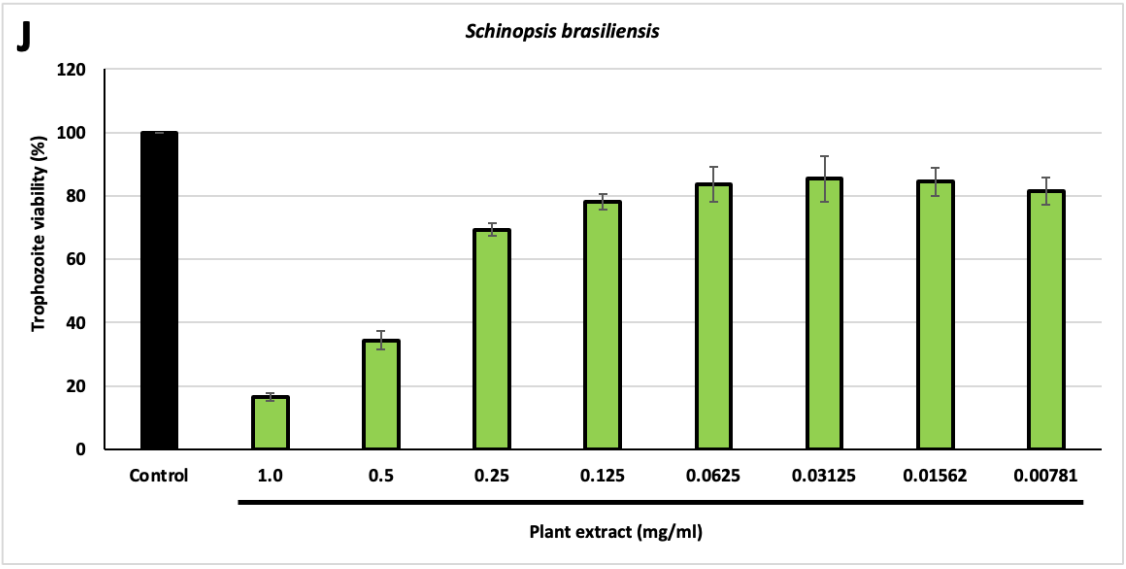

F
Hymenaea courbaril
Trophozoite viability (%)
120
100
80
60
40
20
0
Control
1.0
0.5
0.25
0.125
0.0625
0.03125
0.01562
0.00781
Plant extract (mg/ml)

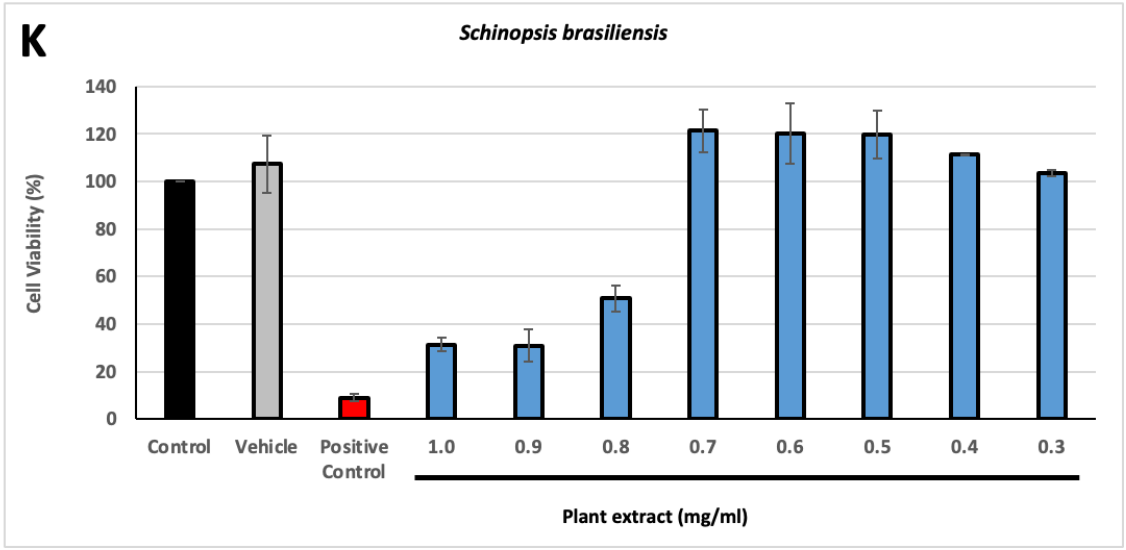

G
Hymenaea courbaril
Cell Viability (%)
140
120
100
80
60
40
20
0
Control
Vehicle
Positive Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Plant extract (mg/ml)

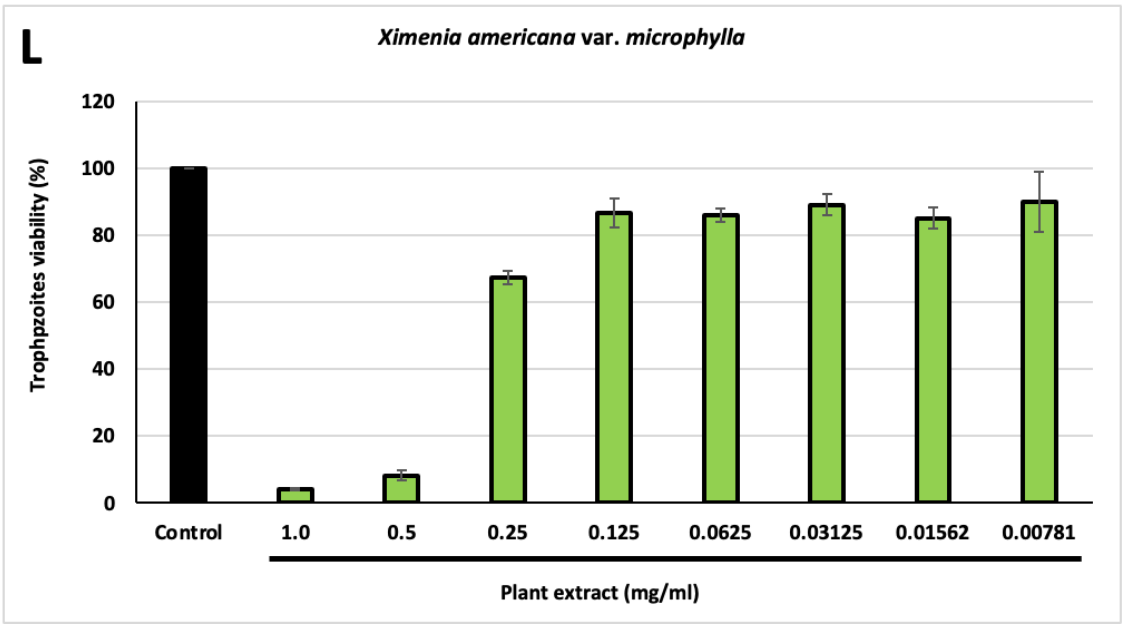

H
Myracrodruon urundeuva
Trophozoite viability (%)
120
100
80
60
40
20
0
Control
1.0
0.5
0.25
0.125
0.0625
0.03125
0.01562
0.00781
Plant extract (mg/ml)


I
Myracrodruon urundeuva
Cell Viability (%)
120
100
80
60
40
20
0
Control
Vehicle
Positive Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Plant extract (mg/ml)

I


J
Schinopsis brasiliensis
Trophozoite viability (%)
120
100
80
60
40
20
0
Control
1.0
0.5
0.25
0.125
0.0625
0.03125
0.01562
0.00781
Plant extract (mg/ml)


K
Schinopsis brasiliensis
Cell Viability (%)
140
120
100
80
60
40
20
0
Control
Vehicle
Positive Control
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Plant extract (mg/ml)



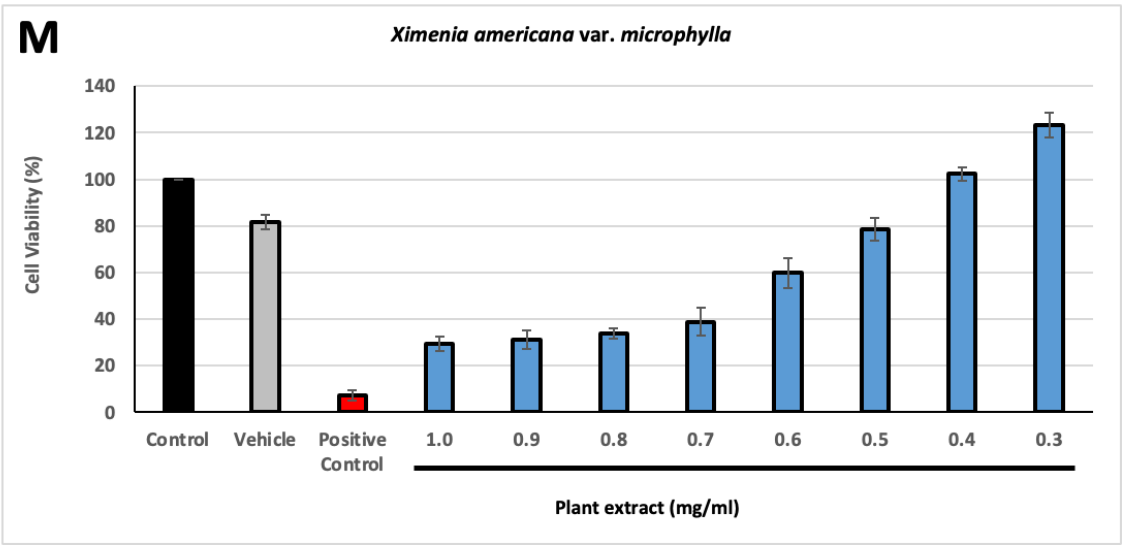


Figure S1. Determination of IC_{50} values for anti-*Tritrichomonas foetus* activity (graphs B, D, F, H, J, L), except for *Cedrela* spp., which extract had IC_{50} values determined for anti-*Trichomonas vaginalis* and anti-*Tritrichomonas foetus* activities (graphs A and B, respectively). The *T. vaginalis* ATCC 30236 isolate, and *T. foetus* TFK isolate were cultured in TYM medium (trypticase-yeast extract-maltose) pH 6.0 and 7.2, respectively, supplemented with 10% inactivated bovine serum. The screening was performed in 96-well microplates, the plant extracts concentration used was 1.0 mg/mL and the trophozoites were added at final density of 2.0 $x10^5$/mL, maintained at 37 °C for 24 h in 5% CO_2. Two controls were conducted: parasites only and metronidazole (100 µM or 0.0171 mg/ml). The activity was determined by assessing the motility and morphology of parasites compared with the negative control by counting in hemocytometer using trypan

blue dye exclusion (0.2%, v/v). Viability was determined as the percentage of viable trophozoites compared to the negative control (100% viability). The active extracts in the screening assay had the half maximal inhibitory concentration (IC_{50}) value determined with concentrations ranging from 1.0 to 0.0078 mg/mL, by serial dilution. Graphs C, E, G, I, K, M show the cytotoxicity of plants extracts tested against human vaginal epithelial cells (HMVII lineage) cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum and 25 µg/mL penicillin at 37 °C and 5% CO_2. Briefly, $1.5x10^4$ cells per well at fifteenth passage were seeded in 96-well microplates for 48 h; the medium was replaced with fresh medium containing or not (control condition) active extracts at IC_{50} concentration range (1.0 - 0.3 mg/ml). Triton X-100 0.2% was used as a positive control. The plates were incubated for 48 h. After this time, a solution of 3-(4,5-dimethylthiazol-2-yl)− 2,5-diphenylte-trazolium bromide (MTT) (0.5 mg/mL) was added and incubated for 1 h at 37 ◦C. MTT was removed and the insoluble purple formazan was dissolved in dimethyl sulfoxide (DMSO). The amount of reduced MTT was measured at 570 nm. Graphs show CC_{50} estimate using GraphPadPrism6 software through a non-linear regression model. Bars represent cell viability as mean ± standard deviation obtained by MTT assay.