Supplementary Information

Bioassay-based *Corchorus capsularis* L. leaf-derived β-sitosterol exerts antileishmanial effects against *Leishmania donovani* by targeting trypanothione reductase

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1. Supplementary Result

Assessment of cytotoxicity of *Corchorus capsularis* L. leaf extract. Cytotoxic effect of chloroform extract of *Corchorus capsularis* L. leaf was investigated against host macrophages by MTT assay method and dose response graph demonstrated that only 11.29±0.37% cells with reference to control were affected with the highest dose (1000 µg/ml) of the leaf extract even after 48 h of treatment (Supplementary Fig. S6). Furthermore, the particular dose at which 50% of promastigotes is deceased as shown in our previous report[1], that dose was safe on host macrophages.

2. Supplementary Method

Cytotoxicity assay. In order to check the toxic effect of chloroform extract of *Corchorus capsularis* L. leaf on host murine macrophages, RAW 264.7 macrophages cell line was maintained in RPMI 1640 supplemented with 10% FCS (Gibco) in addition of 100 U/ml penicillin and 100 mg/ml streptomycin at 37ºC in 5% CO_2 atmosphere[2]. Afterwards, viability of macrophages was assessed by previously described trypan blue (Sigma-Aldrich) exclusion method[3]. Then, RAW 264.7 macrophages (1×10^5/ml) were adhered into a 96-well plate (BD falcon) and treated with the extract at the concentration dose ranging from 0 to 1000 µg/ml for 48 h. After 48 h of treatment MTT was added then amount of formazan produced which is directly proportional to the number of metabolically active cells was finally measured at 570 nm in iMark Microplate Reader (Bio-Rad).

3. Supplementary Figures

Figure S1.

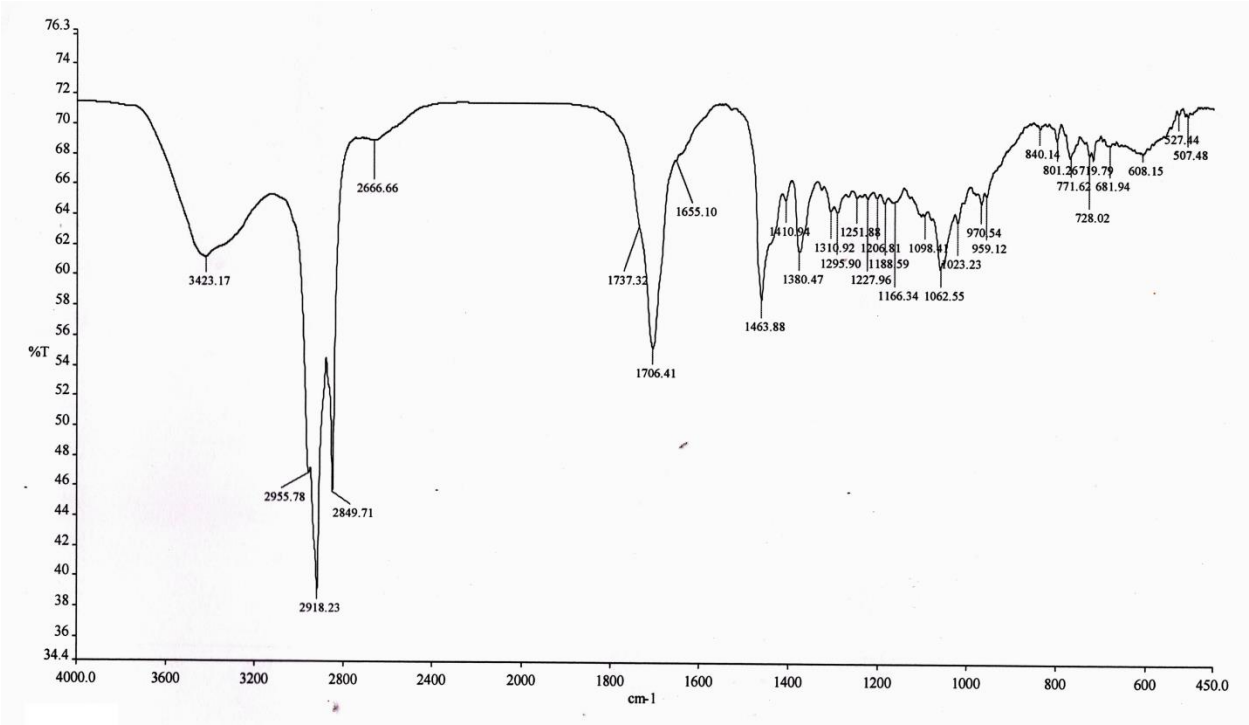

Figure S1. FTIR absorption spectrum of *Corchorus capsularis* L. leaf derived β-sitosterol (β-sitosterol$_{CCL}$). The Spectrum was recorded in Perkin Elmer FTIR spectroscopy ranging from 450 to 4000 cm^{-1}.

Figure S2.

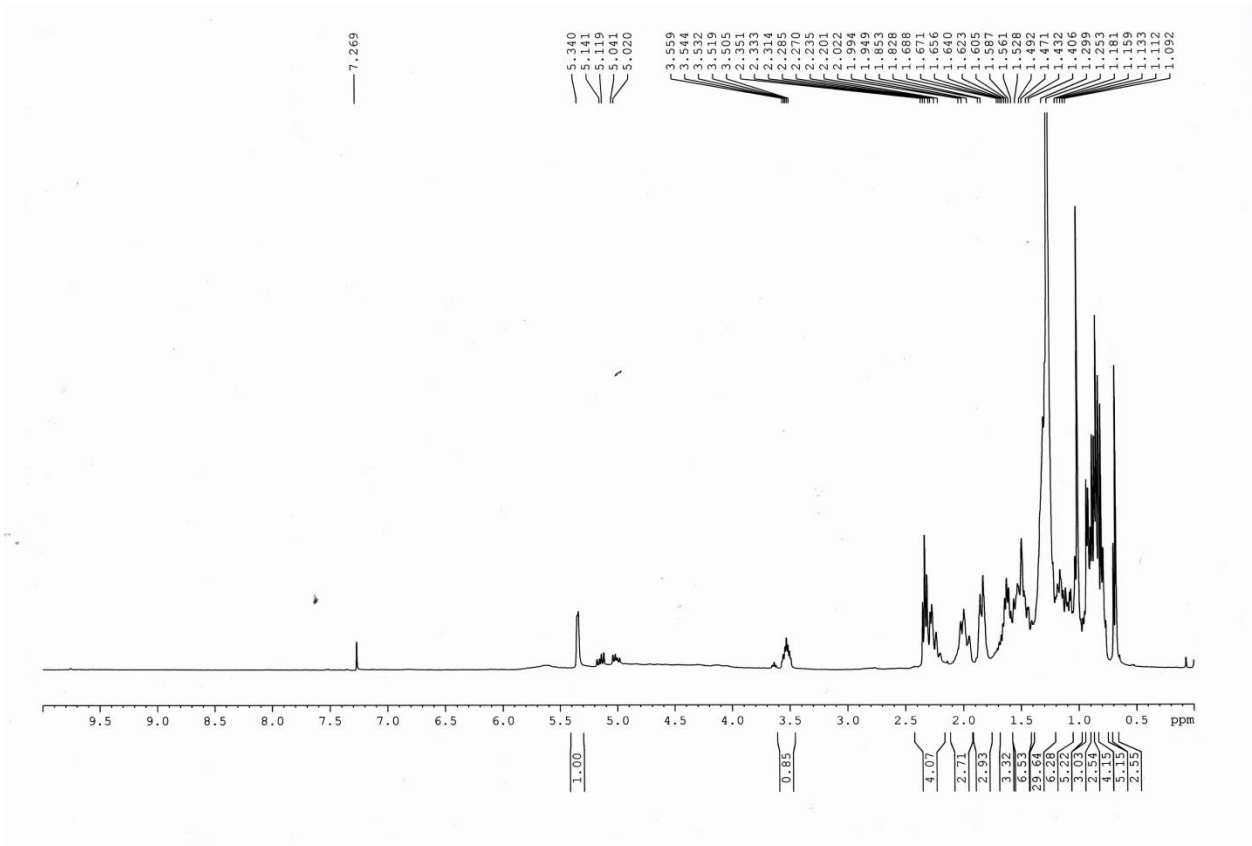

Figure S2. ^{1}H NMR spectrum of *Corchorus capsularis* L. leaf derived β-sitosterol (β-sitosterol$_{CCL}$). The NMR spectrum was recorded in a Bruker Avance spectrometer at 400 MHz by using $CDCl_3$ as solvent system.

Figure S3.

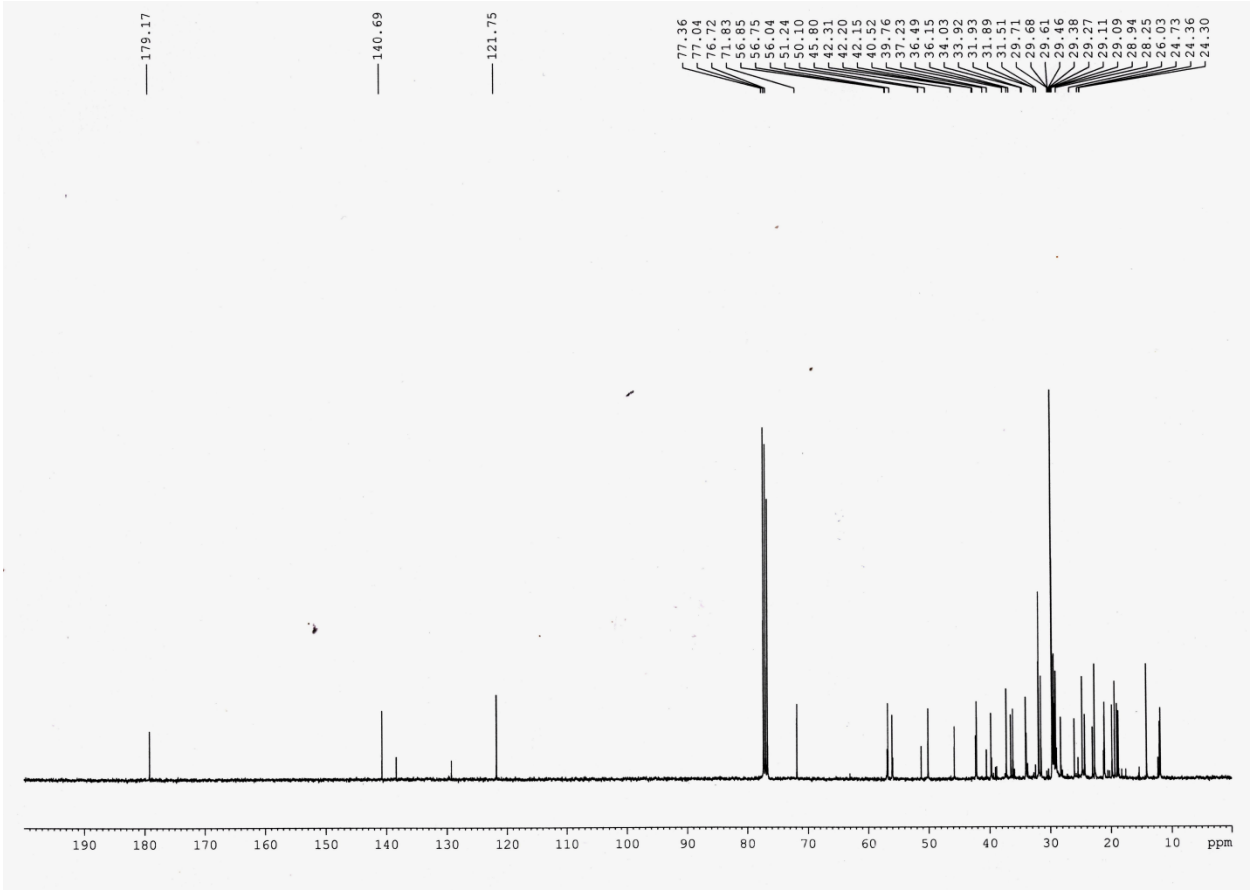

Figure S3. ^{13}C NMR spectrum of *Corchorus capsularis* L. leaf derived β-sitosterol (β-sitosterol$_{CCL}$). The spectrum was recorded in a Bruker Avance spectrometer at 100 MHz by using $CDCl_3$ as solvent system.

Figure S4.

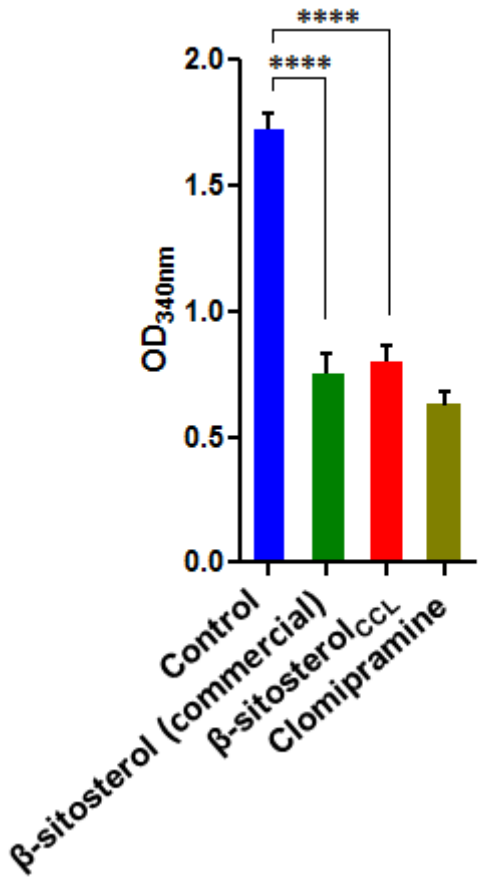

Figure S4. TryR assay in soluble extract of *L. donovani* promastigotes. Effect of *Corchorus capsularis* L. leaf derived β-sitosterol (β-sitosterol$_{CCL}$) on the activity of TryR enzyme in soluble extract of *L. donovani* promastigotes was evaluated by measuring NADPH consumption compared to control. Herein, commercial β-sitosterol (Abcam, USA) was used to validate the observation of β-sitosterol$_{CCL}$. Clomipramine (10 μM) was used as positive control. Results are representative of three separate experiments of mean±S.E. and statistical significance is calculated compared to control by using one-way ANOVA with Dunnett's multiple comparison test; where, ****$p < 0.0001$ is considered as statistically significant.

Figure S5.

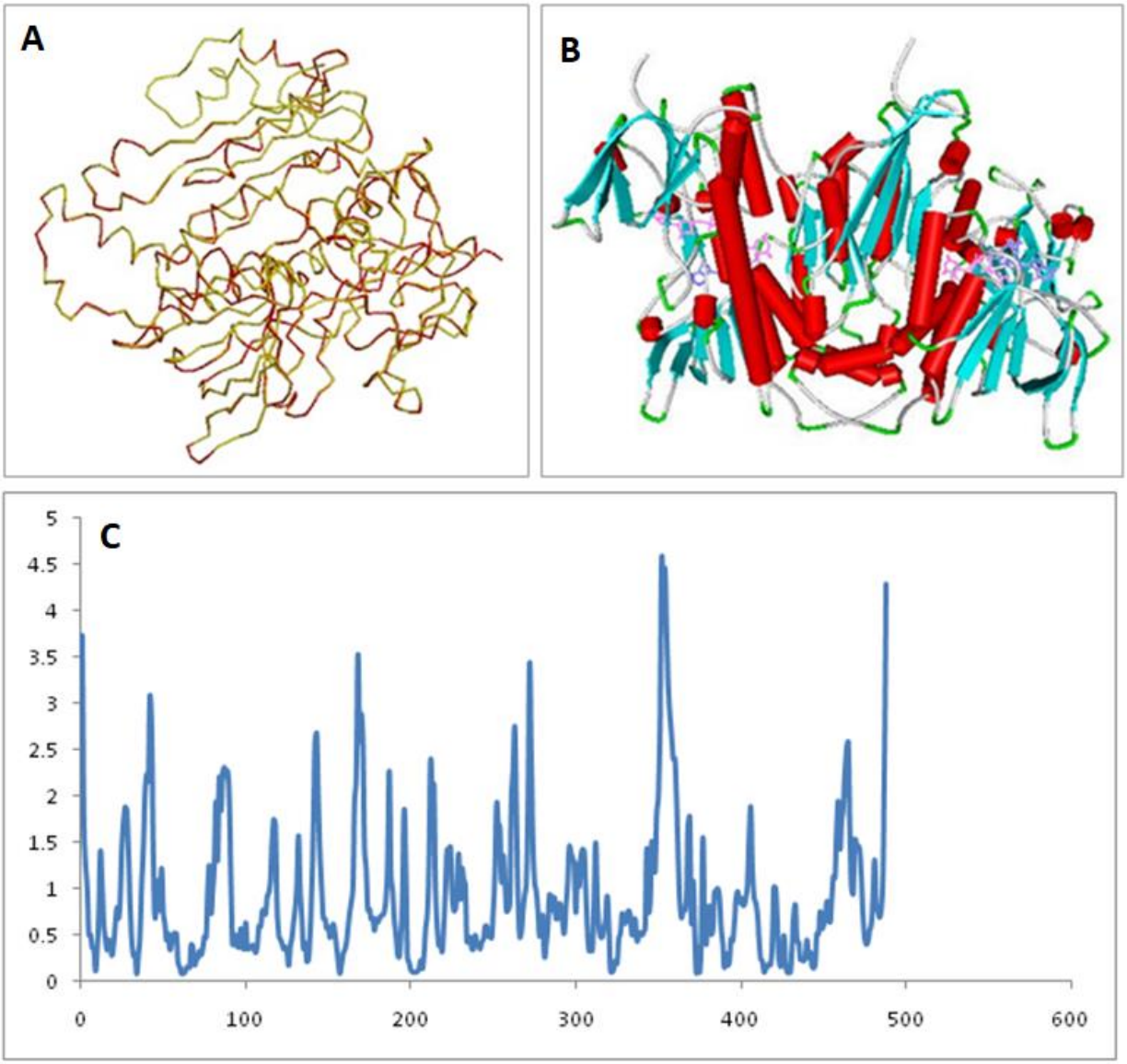

Figure S5. (A) Superimposition of the backbones of *Ld*TryR against the template. *Ld*TryR is presented in red and the template is presented in yellow. The RMSD of the backbone atoms of the *Ld*TryR with template was 0.25Å reflecting a good model quality. (B) Built 3D modeled structure of homodimeric *Ld*TryR along with FAD molecule (pink) and NADPH molecule (blue). (C) Extent of fluctuations of the dimeric *Ld*TryR. The most fluctuating amino acid residues are Pro42, Asp84, Asp142, Gly168, Asp272, Gly352, Ser464, Ser488.

Figure S6.

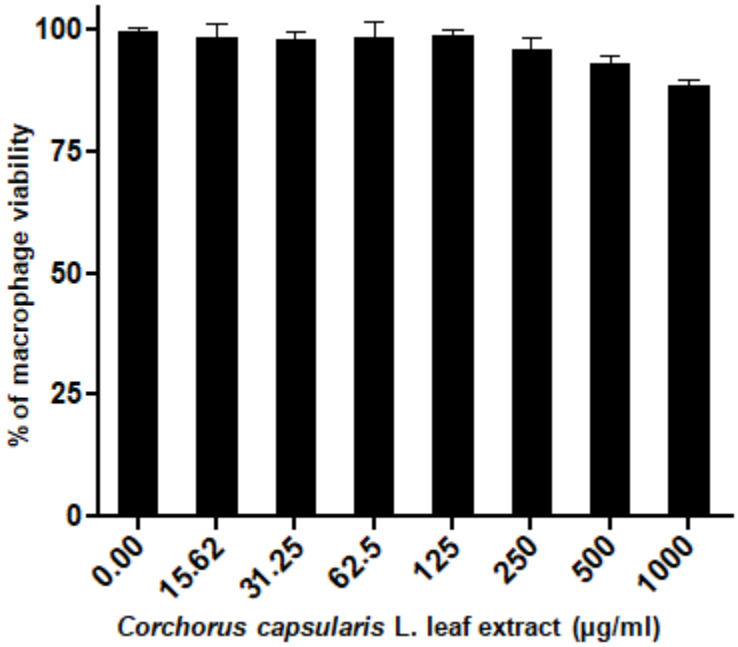

Figure S6. Cytotoxic effect of *Corchorus capsularis* L. leaf extract against murine RAW 264.7 macrophages. Macrophages (1×10^5/ml) were treated with increasing concentration of the extract (0-1000 µg/ml) for 48 h, MTT assay was performed and 11.29±0.37% cells were found to be affected with highest dose (1000 µg/ml) reference to control. The results are expressed herein from three independent experiments as mean ± S.E.

Figure S7.

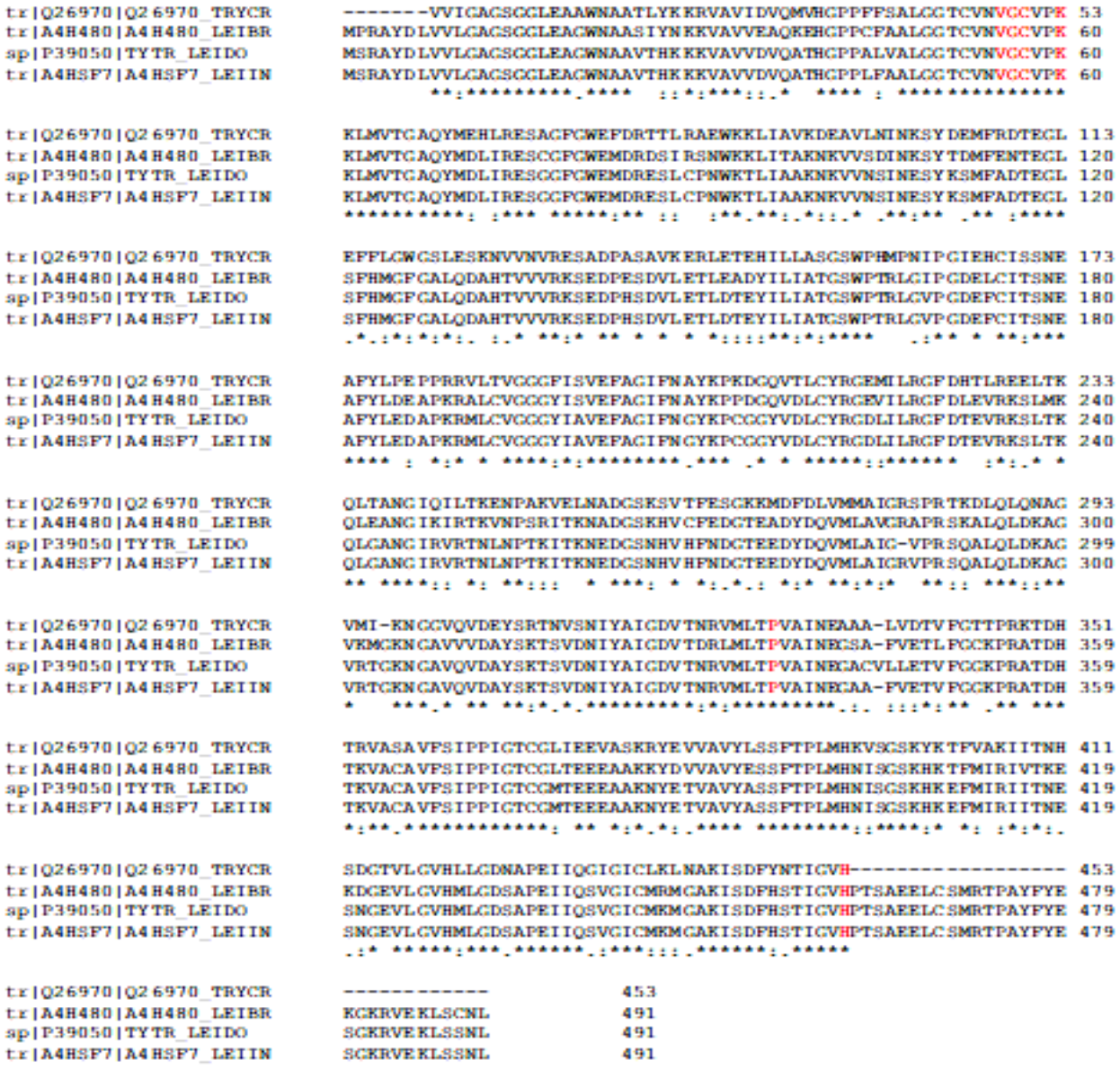

Figure S7. Multiple sequence alignment method. Multiple sequence alignment was performed by using the amino acid sequences of the proteins *LiTryR* from *Leishmania infantum* TryR, *Tc*TryR from *Trypanosoma cruzi* TryR and *Lb*TryR from *Leishmania braziliensis* TryR. The identified active site amino acid residues are marked in red.

4. Supplementary Tables

Table S1. Description and effect of fractions (F1-F13) on *L. donovani* promastigotes.

Major Fraction	Pooled fraction	[a]IC_{50} (µg/ml)
F1	1-15	58.4±2.14
F2	16-65	47.3±1.67
F3	66-84	105.6±4.91
F4	85-89	17.7±0.43
F5	90-91	21.43±0.82
F6	92-109	37.2±1.60
F7	110-143	31.2±1.70
F8	144-171	55.5±2.05
F9	172-195	72.8±0.78
F10	196-208	141.6±1.76
F11	209-230	>200.0
F12	231-308	91.8±2.87
F13	309-441	>200.0

[a]The concentration of fractions that inhibited 50% growth of the *L. donovani* promastigotes. Results are depicted herein as mean ± S.E. of three different independent experiments.

Table S2. List of amino acid residues of homodimeric *Ld*TryR with their binding interaction energy values with the ligand β-sitosterol (β-sitosterol$_{CCL}$) in presence of FAD and NADPH. The first letter represents the chain ID followed by the three letter code of the amino acid and then the residue number of the amino acid of *Ld*TryR. Marked in red are the active site amino acid residues.

Residue	Interaction Energy (kcal/mol)
A_GLY459	-1.053530
A_VAL460	-1.626960
A_HIS461	-3.616330
A_PRO462	-0.970229
A_THR463	-0.196896
B_THR51	-3.366660
B_CYS52	-3.030530
B_VAL55	-2.448090
B_GLY56	-2.219040
B_CYS57	-2.952030
B_LYS60	-0.599220
B_SER162	-1.493710
B_TRP163	-1.055020
B_PRO164	-2.029550
B_THR177	-0.316481
B_SER178	-2.057590
B_ASN179	-1.536950
B_PHE182	-0.675878
B_TYR198	-3.227310
B_ILE199	-2.034010
B_GLU202	-1.991880
B_PHE203	-0.300822
B_MET282	-0.137236
B_ALA284	-0.101688
B_ILE285	-0.587216
B_GLY286	-0.369577
B_VAL287	-0.150055
B_ASP326	-0.828915
B_MET332	-1.991700
B_LEU333	-3.486890
B_THR334	-2.358500
B_PRO335	-1.878500
B_VAL362	-0.047897
B_ALA363	-1.868340
B_CYS364	-1.914780
B_ALA365	-3.463620
B_VAL366	-0.437207
B_PHE367	-1.244690

5. Supplementary References

1. Pramanik, P.K., Paik, D., Pramanik, A. & Chakraborti, T. White jute (Corchorus capsularis L.) leaf extract has potent leishmanicidal activity against Leishmania donovani. *Parasitol. Int.* **71**, 41-45 (2019).

2. Das, P., Paik. D., Naskar, K. & Chakraborti, T. Leishmania donovani serine protease encapsulated in liposome elicits protective immunity in experimental visceral leishmaniasis. *Microbes Infect.* **20**, 37-47 (2018).

3. Weidenfeld, I. et al. Homogentisic acid-derived pigment as a biocompatible label for optoacoustic imaging of macrophages. *Nat. Commun.* **10**, 5056 (2019).