### **Supplementary Information**

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

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#### **1. Supplementary Result**

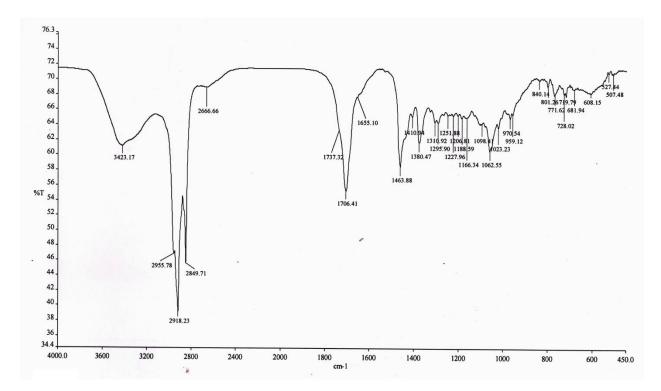
Assessment of cytotoxicity of Corchorus capsularis L. leaf extract. Cytotoxic effect of chloroform extract of *Corchorus capsularis* L. leaf was investigated against host macrophages by MTT assay method and dose response graph demonstrated that only  $11.29\pm0.37\%$  cells with reference to control 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<sup>1</sup>, 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 37°C in 5% CO<sub>2</sub> atmosphere<sup>2</sup>. Afterwards, viability of macrophages was assessed by previously described trypan blue (Sigma-Aldrich) exclusion method<sup>3</sup>. Then, RAW 264.7 macrophages (1×10<sup>5</sup>/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).

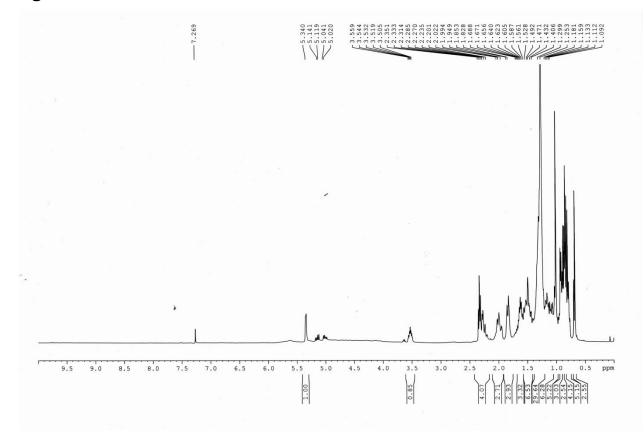
## 3. Supplementary Figures

Figure S1.



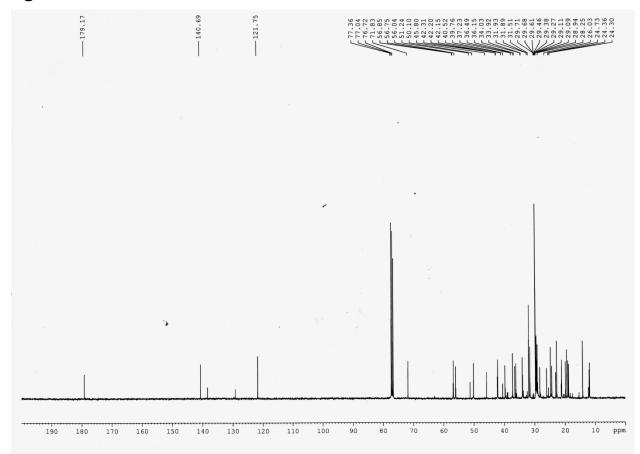
**Figure S1.** FTIR absorption spectrum of *Corchorus capsularis* L. leaf derived  $\beta$ -sitosterol ( $\beta$ -sitosterol<sub>CCL</sub>). The Spectrum was recorded in Perkin Elmer FTIR spectroscopy ranging from 450 to 4000 cm<sup>-1</sup>.

Figure S2.



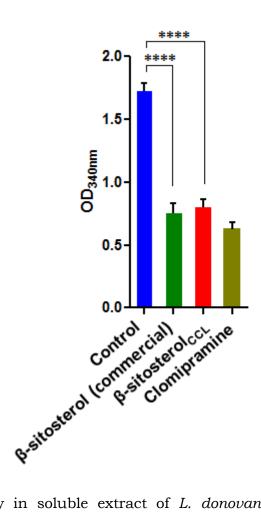
**Figure S2.** <sup>1</sup>H NMR spectrum of *Corchorus capsularis* L. leaf derived  $\beta$ -sitosterol ( $\beta$ -sitosterol<sub>CCL</sub>). The NMR spectrum was recorded in a Bruker Avance spectrometer at 400 MHz by using CDCl<sub>3</sub> as solvent system.

Figure S3.



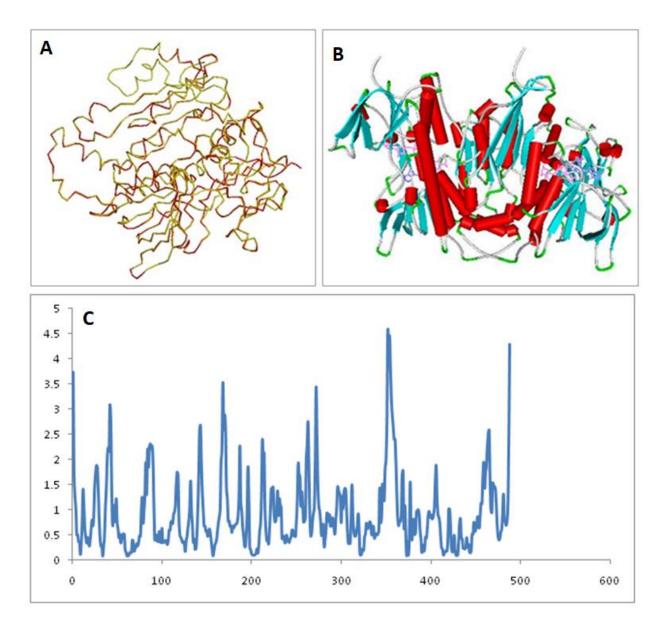
**Figure S3.** <sup>13</sup>C NMR spectrum of *Corchorus capsularis* L. leaf derived  $\beta$ -sitosterol ( $\beta$ -sitosterol<sub>CCL</sub>). The spectrum was recorded in a Bruker Avance spectrometer at 100 MHz by using CDCl<sub>3</sub> as solvent system.

Figure S4.



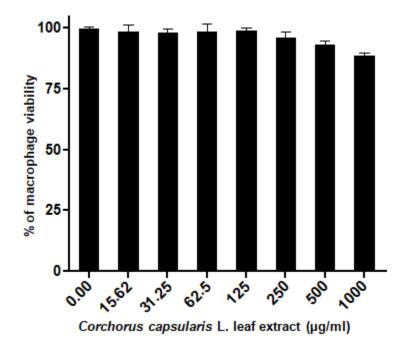
**Figure S4.** TryR assay in soluble extract of *L. donovani* promastigotes. Effect of *Corchorus capsularis* L. leaf derived  $\beta$ -sitosterol ( $\beta$ -sitosterol<sub>CCL</sub>) on the activity of TryR enzyme in soluble extract of *L. donovani* promastigotes was evaluated by measuring NADPH consumption compared to control. Herein, commercial  $\beta$ -sitosterol (Abcam, USA) was used to validate the observation of  $\beta$ -sitosterol<sub>CCL</sub>. Clomipramine (10  $\mu$ M) 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.** (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  $(1 \times 10^5/\text{ml})$  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.

tr Q26970 Q26970 TRYCR	VVIGAGSGGLEAAWN	AATLYK KRVAVI DVQMVHGPPFFSALGG TCVNVGCVPK	5.3
tr A4H480 A4H480_LEIBR	MPRAYD LVVLGAGSGGLEAGWN	AAS I YN KKVAVVEAQKEHGPPCFAALGG TCVNVCCVPK	60
sp[P39050]TYTR LEIDO	MSRAYD LVVLCACSCCLEACWN	AAVTHEREVAVVDVQATHGPPALVALGGTCVNVGCVPE	60
tr A4HSF7 A4HSF7 LEIIN	MSRAYD LVVLCACSCCLEACWN	AAVTHK KKVAVVDVOATHGPPLFAALGG TCVNVGCVPK	60
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tr 026970 026970 TRYCR	KLMUTC MOVMERLIRES ACECWE	FORTTL RAEWKKLIAVKDEAVLNINKSY DEMFRDTEGL	113
tr  A4H480  A4H480 LEIBR		MDRDSIRSNWKKLITAKNKVVSDINKSYTDMFENTEGL	
sp P39050 TYTE LEIDO		MDRESL CPNWKTLIAAKNKVVNSINESY KSMFADTEGL	
tr A4HSF7 A4HSF7_LEIIN		MDRESLCPNWKTLIAAKNKVVNSINESYKSMFADTEGL	120
		*** ** ********************************	
+-10260701026070 BDW0D	PDDI (MOOT DOWNSDAMDROAD)	PASAVKERLETEHILLASGSWPHMPNIPGIEHCISSNE	
tr   Q26970   Q26970_TRYCR			
tr A4H480 A4H480_LEIBR		PESDVLETLEADYILIATGSWPTRLGIPGDELCITSNE	
sp[P39050]TYTR_LEIDO		PHSDVLETLDTEYILIATGSWPTRLGVPGDEFCITSNE	
tr  A4HSF7  A4HSF7_LEIIN		PHSDVLETLDTEYILIATCSWPTRLCVPGDEFCITSNE	180
	.*.:*:*:*:. :.* **:* *	• • • • • •::::••:•:••••	
tr Q26970 Q26970_TRYCR		FAGI FN AYKPKDCQVTLCYRCEMI LRGF DHTLREELTK	
tr A4H480 A4H480_LEIBR		FAGIFN AYKPPDGQVDLCYRGEVILRGF DLEVRKSLMK	
sp[P39050]TYTR_LEIDO	AFYLEDAPKRMLCVGGGYIAVE	FAGI FN GYKPCGGYVDLCYRGDLI LRGF DTEVRKSLTK	240
tr   A4HSF7   A4 HSF7_LEIIN		FAGI FN GYKPCGGYVDLCYRGDLI LRGF DTEVRKSLTK	240
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tr Q26970 Q26970_TRYCR	<b>QLTANG IQILTKENPAKVELNA</b>	DGSKSV TFESGKKMDFDLVMMAIGRSPR TKDLQLQNAG	293
tr A4H480 A4H480_LEIBR	QLEANG IKIRTKVNPSRITKNA	DGSKHV CFEDGTEADYDQVMLAVGRAPR SKALQLDKAG	300
sp   P39050   TYTR_LEIDO	QLCANG IRVRTNLNPTKITKNE	DGSNHVHFNDGTEEDYDQVMLAIG-VPR SQALQLDKAG	299
tr   A4HSF7   A4HSF7 LEIIN	QLCANG IRVRTNLNPTKITKNE	DGSNHVHFNDGTEEDYDQVMLAIGRVPR SQALQLDKAG	300
	** ****:: *: **::: *	***: * *::*:: *:* **:*:* **::: ***::	
tr Q26970 Q26970_TRYCR	VMI-KNGGVQVDEYSRTNVSNI	YAIGDV TNRVMLTPVAINEAAA-LVDTV FGTTPRKTDH	351
tr A4H480 A4H480 LEIBR	VKMCKN GAVVVDAYSKTSVDN I	YAIGDV TDRLMLTPVAINEGSA-FVETL FGCKPRATDH	359
sp   P39050   TYTR LEIDO	VRTCKN CAVQVDAYSKTSVDNI	YAIGDV TNRVMLTPVAINEGACVLLETV FCCKPRATDH	359
tr A4HSF7 A4HSF7 LEIIN	VRTCKN CAVOVDAYSKTSVDNI	YAIGDV TNRVMLTPVAINEGAA-FVETV FCCKPRATDH	359
		*******;*;*********;;; ;;;*;** ;** ***	
tr Q26970 Q26970_TRYCR	TRVASAVFSIPPIGTCGLIEEV	ASKRYE VVAVYLSSFTPLMHKV9CSKYK TFVAKI I TNH	411
tr A4H480 A4H480 LEIBR	TRVACAVFSIPPICTCCLTEEE	AAKKYD VVAVYESSFTPLMEN I SCSKEK TEMIRI VTKE	419
sp P39050 TYTE LEIDO	TEVACA VESIPPICTCONTEEE	AAKNYE TVAVYASSFTPLMHNISGSKHK EFMIRIITNE	419
tr A4HSF7 A4HSF7 LEIIN	TRVACA VFS1PP1CTCCMTEEE	AAKNYE TVAVYASSFTPLMENISCSKEK EFMIRIITNE	419
	*.**.************	***************************************	
tr   Q26970   Q26970 TRYCR	SDGTVLGVHLLGDNAPEIIQGI	GICLKLNAKISDFYNTIGVH	453
tr   A4H480   A4H480 LEIBR	KDCEVL GVHMLGDSAPE I I OSV	GICMRMGAKISDFHSTIGVHPTSAEELC SMRTPAYFYE	479
sp P39050 TYTE LEIDO		GICMEMGAEISDFHSTIGVHPTSAEELC SMRTPAYFYE	
tr   A4HSF7   A4 HSF7 LEIIN		GICMEMGARISDFHSTIGVHPTSAEELC SMRTPAYFYE	
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		<b></b>	
tr Q26970 Q26970_TRYCR		453	
tr   A4H480   A4H480 LEIBR	KGKRVE KLSCNL	491	
sp P39050 TYTE LEIDO	SCERVERLSSNL	491	
tr  A4HSF7  A4HSF7 LEIIN	SCERVE KLSSNL	491	
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**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

Major Fraction	Pooled fraction	<sup>a</sup> IC <sub>50</sub> (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

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

<sup>a</sup>The concentration of fractions that inhibited 50% growth of the *L. donovani* promastigotes. Results are depicted herein as mean  $\pm$  S.E. of three different independent experiments.

**Table S2.** List of amino acid residues of homodimeric LdTryR with their binding interaction energy values with the ligand  $\beta$ -sitosterol ( $\beta$ -sitosterol<sub>CCL</sub>) 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 LdTryR. Marked in red are the active site amino acid residues.

Residue	Interaction Energy
Teolidue	(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).