

Supplementary data

BBD optimized antioxidants of *Crotalaria candicans* and its nanoconjugates, exert potent *in vivo* anti-biofilm effects against MRSA

Ramya M. Subramani^{a†}, Robert Lotha^{a†}, Bhanuvalli R. Shamprasad^a, Sriram Sridharan^b, Ravichandran Natesan^b, Saisubramanian Nagarajan^{c*}, Arvind Sivasubramanian^{a*}

^aDepartment of Chemistry, School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur, Tamil Nadu, India

^b Centre for Advanced Research in Indian System of Medicine, School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur, Tamil Nadu, India

^c Centre for Research on infectious Diseases, School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur, Tamil Nadu, India

*Corresponding authors

^a Arvind Sivasubramanian, Department of Chemistry, School of Chemical and Biotechnology, SASTRA Deemed To Be University, Thanjavur, Tamil Nadu, India; arvi@biotech.sastra.edu

^cSaisubramanian Nagarajan, Centre for Research on infectious Diseases, School of Chemical and Biotechnology, SASTRA Deemed To Be University, Thanjavur, Tamil Nadu, India; sai@scbt.sastra.edu

†Both authors contributed equally

S.No	Metabolites	Rt (min)	[M+H] + (m/z)	MS/MS (Product Ion)	Ref.
1	Fisetin	10.4-10.9	503.4	285.1, 255.0,163.1,119.1	Mass bank database (BML00306) ¹
2	Kaempferol 3-glucuronide	10.9-11	461.1	285.1,63.2,243.0	Mass bank Database(PR100967) ²
3	Quercetin-3-O Glucuronide	11.9-12	477.1	301.1,249.151.0,478.2	Mass bank database(PR100987) ³
4	Morin	12.2-12.5	603.1	301.1,272,151	Mass bank database(BS003338) ²
5	Kaempferol-3-O-sambubioside	13.5-13.9	579.1	285.1,381.1,441.1	Mass bank database(PM017404) ⁴
6	Quercetin	34.9-35.1	603.1	301.1,151.0,105.3,	Mass bank database(PT204090) ²
7	Quercetin-3-O-(caffeoyl)- glucoside	35.5-36	625.1	301.1,381.1,423.1	Mass bank database ⁵

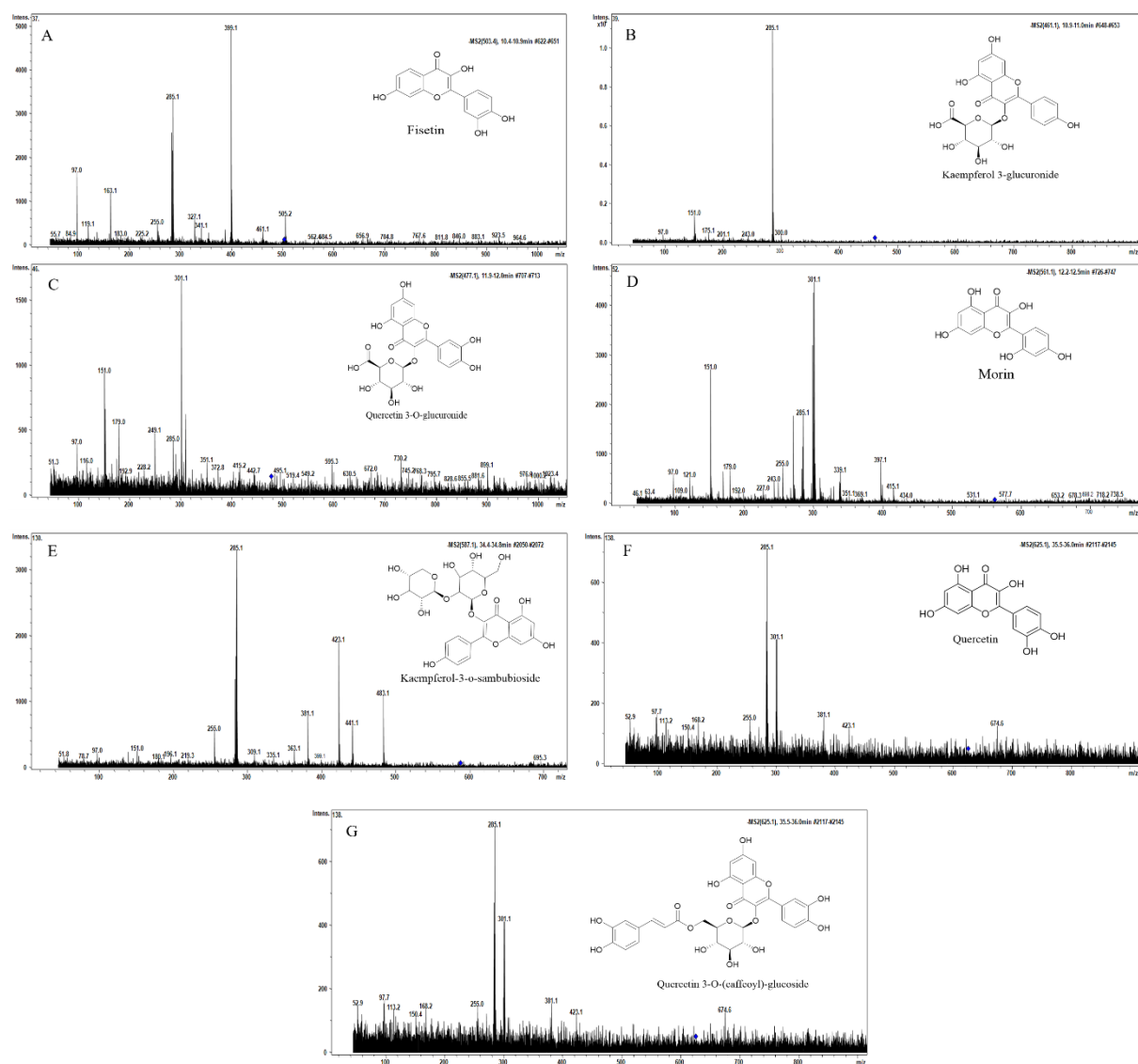


Figure S1: LC-MS/MS spectra of the flavonoids enriched *Crotalaria candicans* fraction.

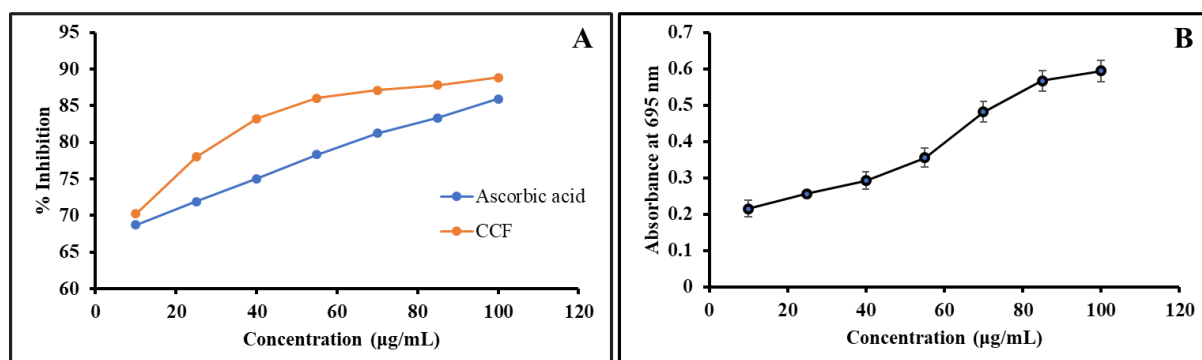


Figure S2: CCF Antioxidant Assay : (A) DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (B) CCF Reducing power

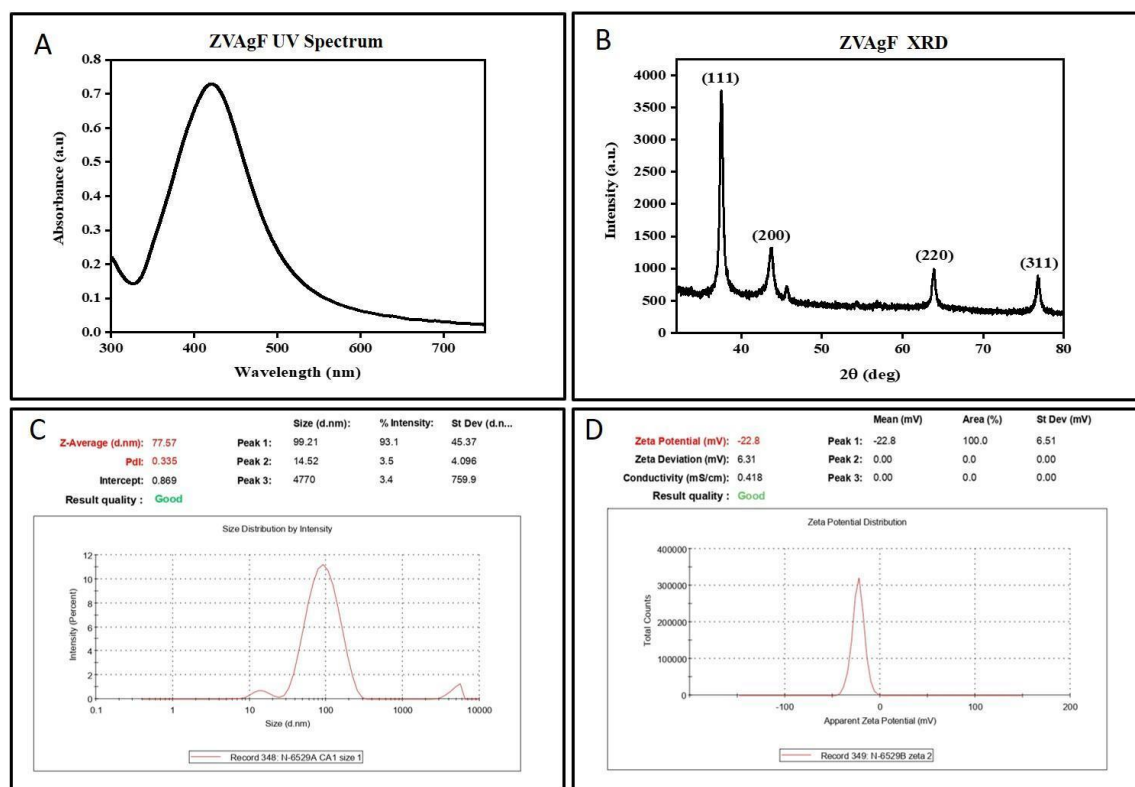


Figure S3: Physicochemical characteristics of ZVAgF (A) UV Spectrum (B) XRD (C) Zeta-sizer (D) Zeta-potential

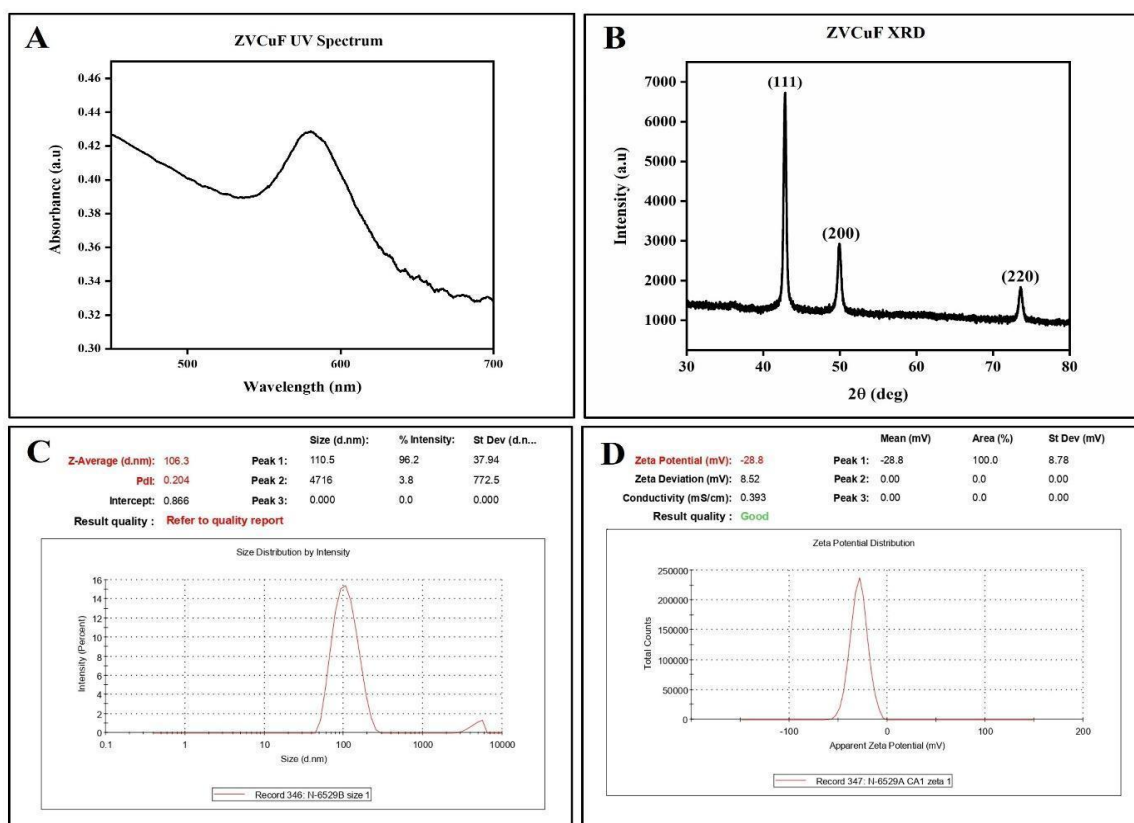


Figure S4: Physicochemical characteristics of ZVCuF (A) UV Spectrum (B) XRD (C) Zeta-sizer (D) Zeta-potential

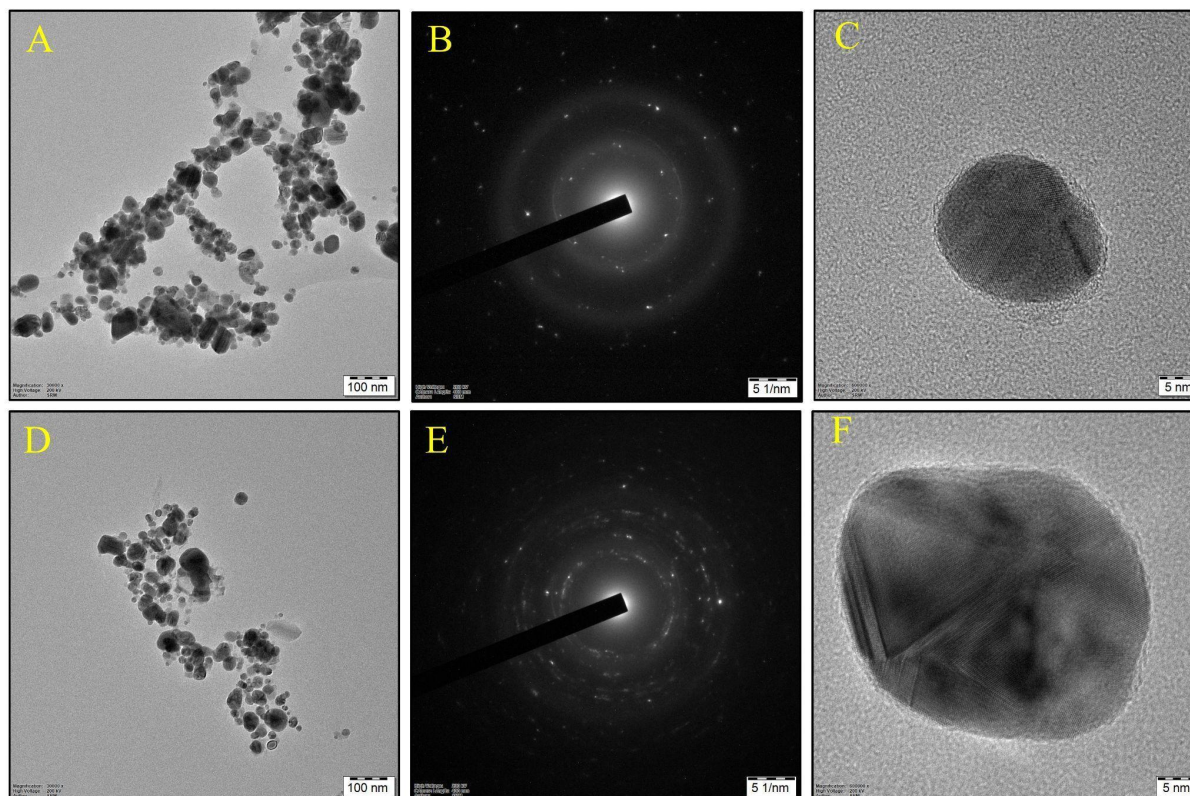


Figure S5: TEM Analysis of ZVAgF (A) 100 nm (B) SAED (C) 5nm; ZVCuF (D) 100 nm (E) SAED (F) 5nm

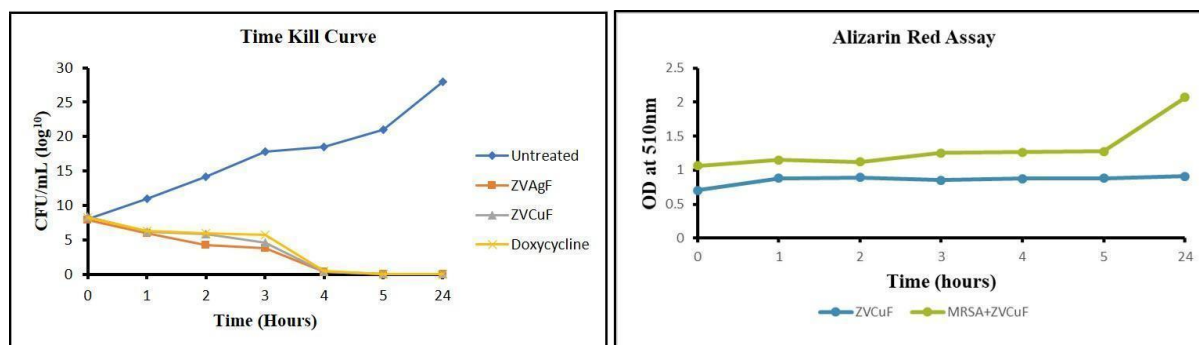


Figure S6. (A) Time kill curve and (B) Alizarin Red Stain (ARS) conjugation test

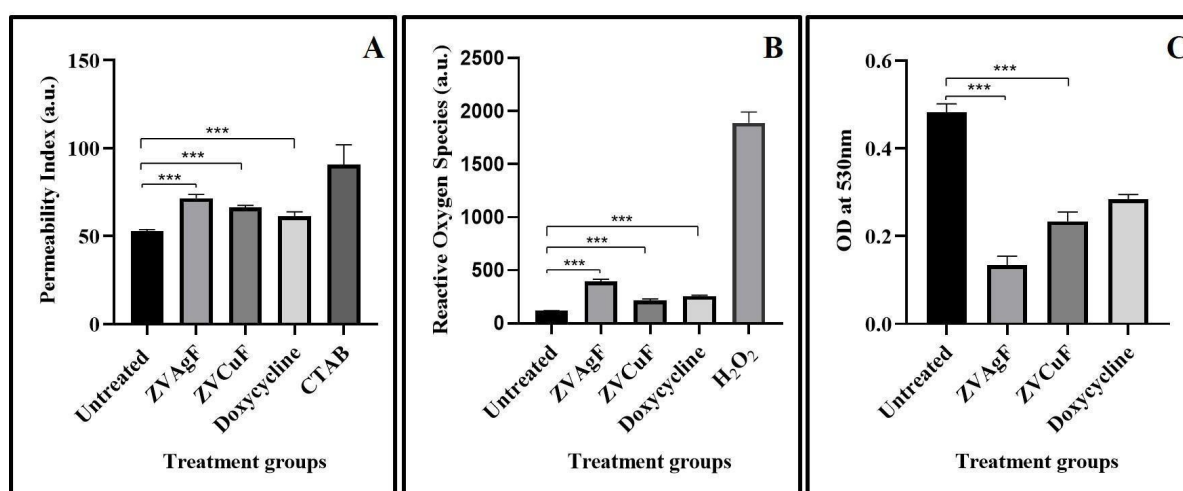


Figure S7: (A) Membrane perturbation, (B) Reactive Oxygen Species and (C) Hydrophobicity Assay

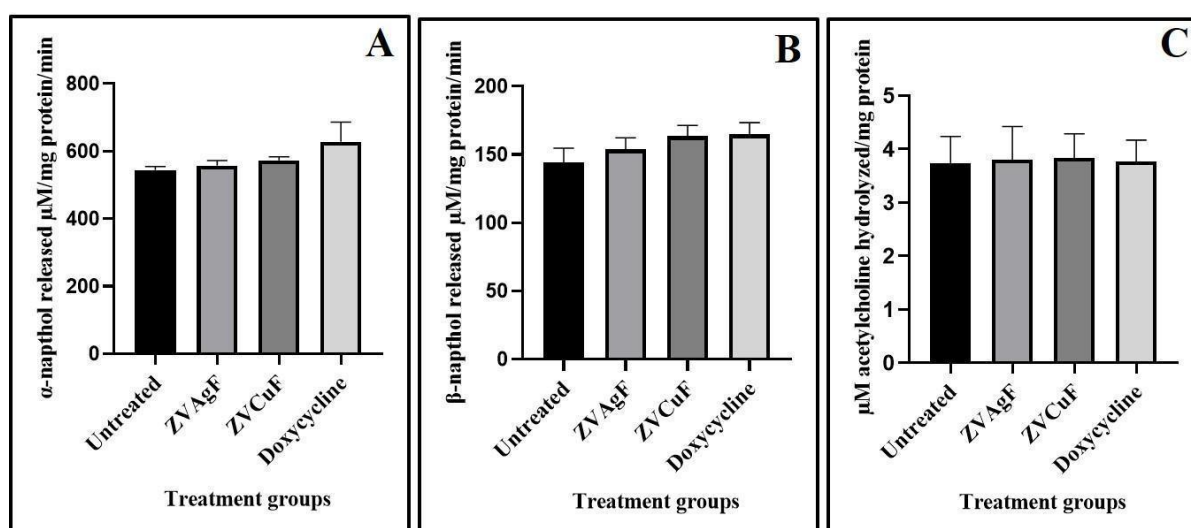


Figure S8: Biogenic CuNPs effect on liver carboxylesterase and brain acetylcholinesterase activity ZVAgF/ZVCuF and Doxycycline on liver carboxylesterase activity A) α-naphthol, B) β-naphthol and on the brain, C) acetylcholinesterase level.

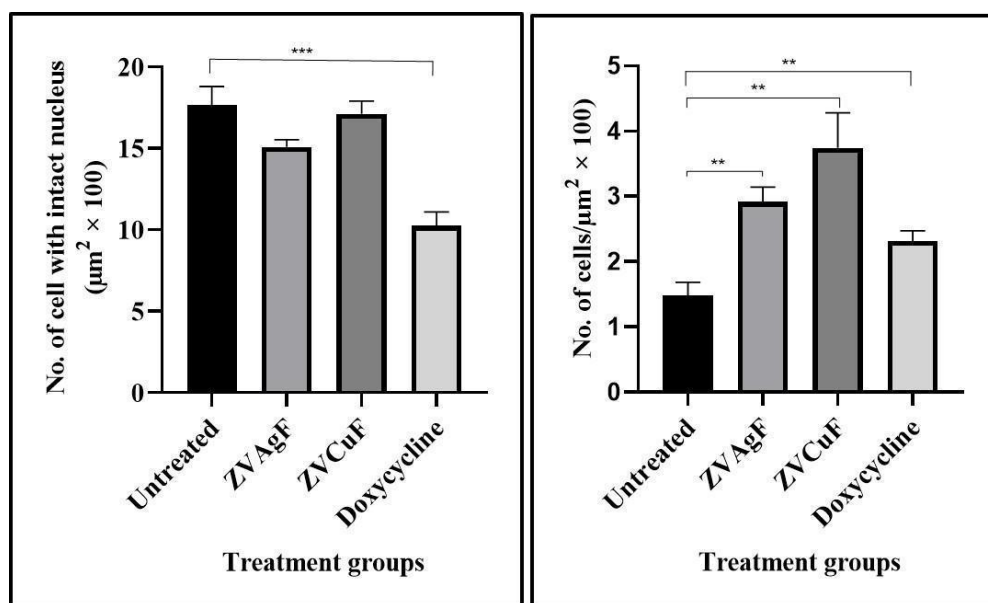


Figure S9: Quantification of normal/ infiltrated cells in zebrafish liver.

Reference

1. Jo, J. H., Jo, J. J., Lee, J.-M. & Lee, S. Identification of absolute conversion to geraldol from fisetin and pharmacokinetics in mouse. *Journal of Chromatography B* **1038**, 95–100 (2016).
2. Chen, Y. K., Chen, S. Q., Li, X. & Zeng, S. Quantitative regioselectivity of glucuronidation of quercetin by recombinant UDP-glucuronosyltransferases 1A9 and 1A3 using enzymatic kinetic parameters. *Xenobiotica* **35**, 943–954 (2005).
3. Day, A. J. *et al.* Human metabolism of dietary flavonoids: Identification of plasma metabolites of quercetin. *Free Radical Research* **35**, 941–952 (2001).
4. Iwashina, T. *et al.* Anthocyanins and flavonols from the blue flowers of six *Meconopsis* species in Bhutan. *Biochemical Systematics and Ecology* **86**, 103925 (2019).

5. Francescato, L. N., Debenedetti, S. L., Schwanz, T. G., Bassani, V. L. & Henriques, A. T. Identification of phenolic compounds in *Equisetum giganteum* by LC–ESI-MS/MS and a new approach to total flavonoid quantification. *Talanta* **105**, 192–203 (2013).