In the recent years, the use of natural products from plants has witnessed a lot of attention as the derived biopesticides also known as secondary metabolites, have shown abilities of killing or repelling mosquitoes (Youmsi et al., 2017; Ali et al., 2023). It has also been found to be target specific, non-toxic to valuable natural enemies, fully biodegradable (Vivekanandhan et al., 2018; Vivekanandhan et al., 2020), of broad spectrum (Ebadollahi et al., 2020), and a promising alternative to synthetic insecticides (Yohana et al., 2022). Their use is envisaged to be sustainable as they are readily regenerative, of low cost and environmentally safe (Borges, 2016; WHO, 2020). The use of extracts of *U. massaica*, though proven as botanicals with promising biospesticide potential (Khatoro et al., 2021; Owiti et al., 2025), the demonstration has not been exhaustive. This study therefore demonstrates in vitro toxicity levels of *Urtica massaica* on *Anopheles gambiae* mosquitoes.

2 Results

It was found that methanol extracts were required in smaller doses as compared to hexane extracts of stem or roots for the LC_{50} regardless of plant parts or immature stage exposed. However, the trend for the LC_{90} was different. Dose did not influence ($p > 0.05$) mortality of exposed aquatic stages (Table 1). For hexane solvent, extracts from the roots were more toxic than those of stem and leaves for LC_{50} regardless of immature stage exposed. No singular trend was observed for the LC_{90}. Dose and solvent of extraction significantly influenced mortality ($p < 0.05$) of all exposed aquatic stages except for L3s exposed to root extract ($p > 0.05$) (Table 2). However, because all observed calculated goodness of fit were greater than the critical value ($\chi^2 = 22.4$; df = 22; $p < 0.05$) for all cases, the null hypothesis was rejected and the alternative adopted.

Table 1 Lethal concentration (LC_{50} & LC_{90}) of methanol extracts of *U. massaica* plant parts against different aquatic stages of *An. gambiae* mosquitoes

Life stage	Plant part	LC_{50}			LC_{90}			Chi-Square Tests		
		Estimate	95% confidence limit for log $_{10}$ (Concentration)		Estimate	95% confidence limit for log $_{10}$ (Concentration)		χ^2	df	p
			Lower	Upper		Lower	Upper			
Eggs	Leaves	4.86[a]	0.42	0.86	48.39[a]	1.46	2.10	269.13	22	0.000
	Stem	4.56[a]	0.60	0.71	19.48[a]	1.22	1.37	49.28	22	0.001
	Roots	11.91[a]	0.92	1.22	50.69[a]	2.33	3.37	98.54	22	0.000
L3	Leaves	1.86[a]	2.20	0.57	9.05[a]	0.68	2.39	901.19	22	0.000
	Stem	-	-	-	-	-	-	-	-	-
	Roots	3.06[a]	0.45	0.52	5.64[a]	0.66	0.75	46.96	22	0.001
Pupae	Leaves	5.22[a]	0.68	0.76	8.61[a]	0.88	1.02	129.74	22	0.000
	Stem	6.12[a]	0.69	0.91	9.33[a]	0.86	1.24	704.08	22	0.000
	Root	12.46[a]	0.46	1.61	21.02[a]	2.93	13.50	175.40	22	0.000

Notes: df = degree of freedom; χ^2 = the chi-square factor; P = probability for the level of significance. P was taken as significant at $p<0.05$; LC = refers to lethal concentration, LC_{50} & LC_{90} concentration that kills 50% & 90% of exposed experimental aquatic stage; L3 = third larval instar. Rows having LC estimates superscripted with letter "a" denotes no significant influence of dose on exposed *An. gambiae* aquatic stages

3 Discussion

In the study herein, it was demonstrated that leaf and root extracts of methanol and hexane extracts respectively were required in smaller amounts and therefore more toxic than extracts of the other parts of *U. massaica* regardless of aquatic stage exposed or dose administered. This could be explained by differential concentration of bioactives in different parts of a plant. Indeed, secondary metabolites also known as botanicals are distributed differently in different plant parts. That is some plant parts have higher and others lower concentrations. This could be judged from the activity of extracts from different parts of a plant in a bioassay. The amount therein being directly proportional to activity. The more the concentration, the more potent the extracts (Yugi and Kiplimo, 2017). It is assumed therefore that the leaves and roots for methanol and hexane extracts contained the highest concentrations of botanicals respectively, a finding that was consistent with those of Anupam et al., (2012) and Yugi and Kiplimo, (2017), for methanol extracts but inconsistent with that of Thouri et al., (2017) for hexane extracts.