Anti-Plasmodial and Larvicidal Effects of Surface Exudates of *Gardenia ternifolia* Aerial Parts

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Abstract: Various parts of *Gardenia ternifolia* (leaves, roots and stem bark) have been reported by traditional healers as a remedy against malaria fever. The aerial parts are coated with shiny materials rich in flavonoid aglycones. The crude acetone wash of the aerial parts showed anti-plasmodial activity of IC_{50} values 1.06 and 0.94 μg mL^{-1} against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum*, respectively. Bioassay guided chromatographic separation of the crude extracts afforded five flavonoids; (naringenin-7-O-methylether, quercetin-4, 7-O-dimethylether, kaempferol-7-O-methylether, 4, 5-Dihydroxy-6, 7-dimethoxyflavanone, naringenin-4, 7-O-dimethyl-ether) and two steroids; (stigmasterol, β-sitosterol). The same crude acetone surface extract as well as the pure isolates showed moderate larvicidal effects against 2nd instar *Ae. aegypti* larvae. Naringenin-7-O-methyl-ether was the most potent principle.

Key words: *Gardenia ternifolia*, surface exudates, naringenin-7-O-methylether, quercetin-4, 7-O-dimethylether, kaempferol-7-O-methylether, anti-plasmadial, LC_{50}, IC_{50}

INTRODUCTION

Malaria is the most prevalent insect borne disease killing between 1-2 million people annually with as many as 300-500 million people being infected. Most of the world population is at risk of infection with fatal rates being high among children <5 years of age. It is common in the poorer and less developed countries of the tropical regions; Africa for instance, faces the greatest impact (WHO, 1990).

Currently, malaria parasite is being managed by both natural and synthetic agents. The use of drugs with botanical origin is being emphasized due to the emergence of drug-resistant *Plasmodium falciparum*. Many of the currently prescribed anti-malarial drugs are becoming less effective (Winstanley *et al.*, 2002).

In Kenya, there has been effort to search for botanical remedy and a number of plants have demonstrated potent anti-malarial activity (Philipson and Wright, 1991; Oketch-Rabah, 1996). Ethno-medical information indicates that *Gardenia ternifolia* (Rubiaceae) has been used by traditional healers to palliate malaria and its related fevers (Kokwaro, 1993; Weenen *et al.*, 1990).

In Nigeria, the fresh and dried fruit extracts are used to manage hemorrhoids lesion and anti diabetic activity (Midiwo *et al.*, 1990). In the quest to chemically survey the constituents responsible any activity reported, the plant aerial parts was realized to be covered with shiny surface exudates. Occurrence of surfaces compounds has been reported in several plant families. However, this is the first time such a feature is reported in the genus *Gardenia*. Many possible functions have been speculated for these exudates including being phytoallexin (Johnson, 1983) and UVA and UVB screening (Agrawal, 1989).

No reports are available on *in vitro* or *in vivo* investigation of the anti-plasmodial activity and other biological activities such as larvicidal activity coupled with phytochemical studies to validate and/or to reveal the medicinal efficacy of the plant. In this communication, researchers report the existence of some flavonoids and steroids. The results provided show that the surface compounds have anti-plasmodial and larvicidal activities.

MATERIALS AND METHODS

General: M.P uncorrect analytical TLC was performed using Merck pre-coated silica gel 60 F_{254} plates. CC on silica gel 60 (70-230 mesh) and sepphadex LH-20. EIMS (TOF) direct inlet 70 eV was used. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (125 MHz) were recorded on ARX 300 (Bruker) using TMS as internal standard. HMBC and HMQC spectra were acquired using standard Bruker software. UV λmax was obtained using MeOH as the solvent and NaOAc, AlCl$_3$/HCl as the shift reagents.

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Plant material: The aerial parts of *Gardenia ternifolia* were collected from Machakos District in Kenya in April 2005. The plant identification was done at the University Herbarium, Department of Botany, University of Nairobi where a voucher specimen was deposited.

Extraction and isolation: The leaves were first acetone washed for 15 sec to remove the surface exudates and avoid extracting the internal components and chlorophyll. When the solvents just turned yellow, it was evaporated *in vacuo* using a rotary evaporator. This process afforded a gummy yellowish 8 g extract. A portion (7 g) of the extract was subjected to Column Chromatography (CC) on (70 g) silica gel eluting with hexane containing increasing amount of (10, 20, 30, 40, 50, 70 and 100%) CH_2Cl_2.

A total of 8 fractions were combined to four fractions on the basis of their TLC profiles. Crystallization of the fraction eluted with 20% CH_2Cl_2 in hexane gave 13 mg of β-sitosterol, while 40% CH_2Cl_2 in hexane fraction gave 21 mg of stigmasterol as white needle crystals after repeated CC and crystallization. Prep-TLC on the mother of the second fraction afforded <5 mg of compound 3, naringenin-4, 7-O-dimethyl-ether: β-sitosterol (1), $C_{29}H_{50}O$, a colourless compound on TLC plate with Rf. 0.8 in CH_2Cl_2/hexane (1:1) and m.p. 133-135^{0}C. EIMS (TOF) (M$^+$) = 414 (70), 255 (50), 329 (60), 396 (60), 273 (40). ^{1}H-NMR (Cd_2Cl_2); 1.88 (m, H-1), 1.83 (m, H-2), 3.50 (m, H-3), 2.27 (m, H-4), 5.36 (m, H-6), 1.54 (m, H-7), 1.99 (m, H-80), 1.51 (m, H-10), 0.68 (s, Me-18), 0.87 (s, Me-19), 0.92 (d, Me-21), 0.84 (s, Me-27), 0.85 (dd, Me-28). ^{13}C-NMR (Cd_2Cl_2); 37.5 (C-1), 31.9 (C-2), 72.04 (C-3), 42.6 (C-4), 141 (C-5), 121.9 (C-6), 31.9 (C-7), 32.1 (C-8), 50.4 (C-9), 36.7 (C-10), 21.4 (C-11), 40 (C-12), 42.6 (C-13), 57 (C-14), 24.5 (C-15), 28.5 (C-16), 56.3 (C-17), 12.1 (C-18), 19.3 (C-19), 40 (C-20), 20 (C-24), 34.2 (C-22), 26.4 (C-23), 51.5 (C-24), 32.2 (C-25), 19 (C-26), 19.6 (C-27), 24.5 (C-28) and 12.2 (C-29). Stigmasterol (2), $C_{29}H_{48}O$, a colourless compound on TLC plate. Rf 0.6 in CH_2Cl_2/hexane (1:1) and m.p. 170-171°C. EIMS (TOF) (M$^+$) = 412 (5), (M$^+$-H$_2$O) = 394 (80), (M$^+$-C$_2$H$_5$) = 273 (5), (M$^+$-methylpently) = 329 (5), (M$^+$-isopropyl + H$_2$O] = 351 (5), (M$^+$-side chain + H$_2$O) = 255 (9).^{1}H-NMR (Cd_2Cl_2); 3.49 (m, H-3), 5.35 (m, H-6), 0.68 (s Me-18), 1.01 (s, Me-19), 0.93 (d, Me-21), 5.24 (m, 22/23-Hs), 0.84 (d, H-26), 0.86 (d, H-27), 0.67 (dd, H-29). ^{13}C-NMR (Cd_2Cl_2); 37.5 (C-1), 31.9 (C-2), 72 (C-30), 42.6 (C-4), 140.2 (C-5), 121.7 (C-6), 31.7 (C-7), 32 (C-8), 50.1 (C-9), 36.7 (C-10), 20.8 (C-11), 39.6 (C-12), 42.6 (C-13), 57.6 (C-14), 24.6 (C-15), 28.5 (C-16), 56.3 (C-17), 12.1 (C-18), 19.2 (C-19), 40.13 (C-20), 20.5 (C-21), 138.04 (C-22), 129.2 (C-23), 51.1 (C-24), 32.1 (C-25), 19 (C-26), 19.20 (C-25), 24.4 (C-28), 12.2 (C-29). 5-Hydroxy-4, 7-O-dimethoxyflavanone (3) $C_{17}H_{16}O_5$ a white solid from hexane/CH_2Cl_2 and Rf. O.45 in 100% CH_2Cl_2. EIMS (TOF) (M$^+$) = 300 (40), RDA = 166 (30), 107 (10). ^{1}H-

NMR (Cd$_2$Cl$_2$), 5.34 (dd, J = 12.9, 2.9Hz, H-2), 2.76 (dd, -17.2, 2.9Hz, H-3α), 3.08 (dd, 13.1, -17.2Hz, H-3β), 12.01 (s, 5-OH). 6.05 (s, H-6), 6.03 (s, H-8), 7.36 (d, 8.3Hz, 2'/6' Hs), 6.93 (d, 8.3Hz, 3'/5' Hs), 3.81 (OCH$_3$, C-7), 3.38 (OCH$_3$, C-4), ^{13}C-NMR (Table 1)

Fraction eluted with 50% CH_2Cl_2 in hexane had two components which were resolved using CC on silica gel that eluted out quercetin -4 7-O- dimethyl ether 429 mg as the first band (yellow) followed by naringenin-7-O-methyl ether (106 mg), both isolated in pure form after crystallization in hexane and CH_2Cl_2: 3, 3', 5-Trihydroxy-4', 7-dimethoxyflavone $C_{17}H_{14}O_7$, yellow amorphous solid from hexane/CH_2Cl_2, m.p. 220-222°C and Rf. 0.5 in 100% CH_2Cl_2. UV λmax nm (log $ε_0$) (MeOH) = 256.5 (4), 315.5 (4); EIMS (TOF) (M$^+$) = 330 (100), (M$^+$-H) = 329 (15), 331 (15), (M$^+$-Me) = 315 (7), (M$^+$-CO) = 302 (10), (M$^+$-COMe) = 287 (10), RDA = 167 (10), 121 (10), 151 (15). ^{1}H-NMR (Cd$_2$Cl$_2$); 3.90 (OMe C-7), 4.01 (OMe C-4'), 11.72 (5-OH), 6.39 (d, J = 2Hz, H-6), 6.50 (d, J= 2Hz, H-8), 7.81 (d, J = 2Hz H-2'), 7.06 (d, 8.7Hz H-5'), 7.77 (dd, J = 8.5, 2Hz H-6'). ^{13}C-NMR (Table 1). 4', 5-Dihydroxy-7-methoxyflavanone (5), $C_{16}H_{14}O_5$, a white a morphous solid from hexane/CH_2Cl_2, m.p. 133-135°C and Rf. 0.3 in 100% CH_2Cl_2. UV λmax (MeOH) nm (log $ε_0$) = 223.5 (4). (α)$_D^{25}$ = +58 (R-configuration). EIMS (TOF) (M$^+$) = 286 (70), (M$^+$-H) = 285 (100), 287 (20), RDA = 167 (70), 120 (100), (M$^+$-15) = 243 (10), (M$^+$-17) = 286 (10). ^{1}H-NMR (Cd$_2$Cl$_2$) 5.36 (dd, J = 12.9, 3Hz, H-2), 3.09 (dd, J = -17.1, 12.9 Hz, H-3α), 2.79 (dd, J = -17.1, 3 Hz, H-3β), 12.07 (s, 5-OH), 6.08 (d, J = 2.4Hz, H-6), 6.04 (d, J = 2.4Hz, H-6), 7.30 (dd, J = 6.5, 2Hz, 2'/6' Hs), 6.80 (dd, 6.5, 2Hz 3'/5' Hs), 3.81 (s OMe C-7). ^{13}C-NMR (Table 1). The mother liquor of compound 4 was further subjected to preparative-TLC (developed in 70% CH_2Cl_2 in hexane) to obtain another compound (6) that appeared

Table 1: ^{13}C (125MHz) NMR data of compounds 3, 4, 5, 6 and 7

Atom	3	5	6	4	7
2	79.01	158.41	79.57	152.82	78.96
3	43.19	137.58	43.43	135.63	43.20
4	196.04	177.58	197.03	176.16	196.02
4a	103.13	105.55	105.00	105.00	105.15
5	164.13	162.64	162.00	160.86	164.15
6	95.08	99.06	131.04	92.25	95.12
7	167.90	167.36	167.00	167.00	168.89
8	94.22	93.44	91.98	97.92	94.25
8a	160.04	160.85	156.53	146.45	162.89
1'	130.87	123.97	123.53	122.92	130.50
2'	125.87*	117.06*	128.32*	110.32	127.95*
3'	114.22**	131.22**	115.13**	146.45	115.68**
4'	144.29	148.06	156.13	147.77	156.13
5'	114.22**	131.22**	115.13**	146.20	115.68**
6'	125.87*	117.06*	128.32*	121.80	127.95*
OMe	55.67	57.11	60.78	56.72	55.68
	55.35		56.49	55.85	

Signals with * or ** are for equivalent carbons: ^{13}C assignments done with HMQC correlations

above compound 4 on the TLC plate. Compound 6 (4', 5-Dihydroxy-6, 7-dimethoxylflavanone 6) was cut carefully from the TLC plate, eluted with 100% CH$_2$Cl$_2$ then crystallized in hexane and CH$_2$CL$_2$ to yield 6 mg white solid: 4', 5-Dihydroxy-6, 7-dimethoxyflavanone C$_{17}$H$_{16}$O$_6$ a white needlelike crystal from hexane/ CH$_2$Cl$_2$ and Rf. 0.6 in 100% CH$_2$Cl$_2$. EIMS (TOF) (M$^+$) = 316 (6), RDA = 197 (5), 121 (100). ^{1}H-NMR (Cd$_2$Cl$_2$) 5.34 (dd, J = 13.9, 3Hz, H-2), 2.78 (dd, J = -17.1, 3 Hz, H-3α), 3.09 (dd, J= -17.1, 13.2 Hz H-3β), 11.96 (s, 5-OH), 6.11 (s, H-8), 7.34 (dd, J = 6.6, 2Hz, 2'/6' Hs), 6.88 (dd, J = 6.6, 2Hz, 3'/5' Hs), 3.76 (OMe C-6), 3.86 (OMe C-7). ^{13}C-NMR (Table 1). CC on sephadex LH-20 followed by crystallization of the eluent from fractions eluted with 70% CH$_2$Cl$_2$ in hexane and 100% CH$_2$Cl$_2$ afforded 31 mg of kaempferol-7-O-methyl ether : 3, 4', 5-Trihydroxy-7-methoxyflavone C$_{16}$H$_{12}$O$_6$, a yellow needlelike crystals from hexane/CH$_2$Cl$_2$, m.p. 158-159°C and Rf. 0.26 in 2% MeOH/CH$_2$Cl$_2$. UV (MeOH) λmax nm (log ε$_0$) = 215 (4), 307.5 (4); UV λmax (MeOH/AlCl3/HCl) = 220 (4), 350 (4). EIMS (TOF); (M$^+$) = 300 (100), (M$^+$-H) = 299 (20), (M$^+$-15) = 284 (5), (M$^+$-CHO) = 271 (40), (M$^+$-COMe) = 257 (30), RDA = 121 (40), 167 (5), 93 (15). ^{1}H-NMR (acetone) 9.06 (br s, 3-OH), 8.09 (br s, 4'-OH), 12.14 (s, 5-OH), 6.70 (d, J = 2Hz H-6), 6.32 (d, J = 2Hz, H), 8.19 (dd, J = 6.9, 2.1Hz, 2'/6' Hs), 7.05 (dd, J =6.9, 2Hz 3'/5' Hs), 3.94 (s, OMe C-7). ^{13}C-NMR (Table 1).

***In vitro* anti-plasmodial assay:** The crude extract and pure compounds were assayed using an automated micro-dilution technique to determine 50% growth inhibition of cultured parasites (Desjardin *et al.*, 1979). Two different strains, chloroquine-sensitive Sierra-Leone (D6) and chloroquine-resistant Indochina (W2) of *Plasmodium falciparum* were grown as described in the literature (Desjardin *et al.*, 1979; Chulay *et al.*, 1983). The samples were serially diluted a cross the plate to provide a range of concentration used to determine IC$_{50}$ values. Plates were incubated in a mixed gas incubator for 24 h (^{3}H) hypoxanthine was to grow for an additional 18 h.

Cells were processed with a plate harvester (TomTec) onto filter paper and washed to eliminate unincorporated isotopes. Filters were measured for activity in a microtiter plate scintillation counter. Data from the counter were imported into a Microsoft Excel spreadsheet which was then imported into an Oracle data base/program to determine IC$_{50}$ values. A minimum of three separate determinations were carried out for each sample.

Larvicidal activity assay: The eggs of *Ae aegypti* (Diptera, cullicidae) were obtained from the Department of Zoology, University of Nairobi. The eggs were flooded with sodium chloride solution (0.8 g dm^{-3}) and left to hatch at 28°C. Twenty second-instar larvae were transferred into Petri-dish containing sodium chloride solution (0.8 g dm^{-3}, 40 mL) solution. The larvae were provided with a small amount of food made from finely ground fish food. Different concentration (200, 100, 50, 25, 12.5, 6.25 mg mL^{-1}) of the test samples in Dimethlysulfoxide (DMSO) were prepared by serial dilution. From each test solution 20 µL was transferred into Petri-dishes containing larvae giving final concentration of 100, 50, 25, 12.5, 6.25, 3.125 µg mL, respectively (Mwangi and Rembold, 1988). Control larvae in all cases received 20 µL of DMSO and some compounds whose Mortality was checked after every 24 h for up to the tenth day. The LC$_{50}$ values were calculated from the mean of the three observations for each test sample using finey probit analysis for quantal data (McLaughlin *et al.*, 1991).

RESULTS AND DISCUSSION

The upper surfaces of leaves of *Gardenia ternifolia* are shiny and evergreen covered with epicuticular secretion. The extraction procedure applied was such that only the surface deposited materials were removed. Fresh leaves and petioles were washed by dipping in acetone for about 15 sec, thus avoiding extraction of the internal tissues compounds and chlorophyll (Midiwo *et al.*, 1990). The remaining plant material was air dried and then ground into powder to be serially re-extracted using CH$_2$Cl$_2$ and methanol. The yields were 8, 21 and 30 g extract, respectively from a plant material of 1.15 kg dry mass. The surface exudates was approximated to be 0.7% w/w. TLC analysis of the crude surface exudates and CH$_2$Cl$_2$ extracts were the similar, hence only surface acetone extract and methanol extract were subjected to comparative bioassay.

Crude acetone (surface exudates) extracts of *Gardenia ternifolia* shown higher potent activity than internal tissues methanol extract against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* at IC$_{50}$ values 1.07 µg mL^{-1} and 0.95 µg mL^{-1}, respectively. Whereas internal tissue methanol extract (Fig. 1) shown low activities (IC$_{50}$ 8.36 and 14.36 µg mL^{-1} against W2 and D6 strains, respectively).

This suggested that the possible potent principles in the aerial parts of *Gardenia ternifolia* were exuded on the surface. However, leaf exudates values were still 10 times lower than the activity observed for the crude extracts of *Artemisia annua* (propagated in Kenya) leaves at IC$_{50}$ 0.089 µg mL (Fig. 1) against chloroquine-resistant strain. Chromatographic partitioning of the surface extracts
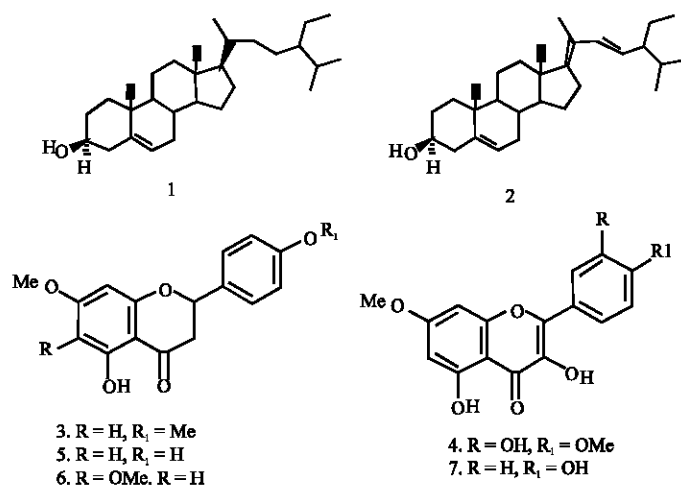
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Fig. 1: Structure of the compounds isolated from *Gardenia ternifolia*

afforded three major fractions which were further assayed against *Plasmodium fulciparum* cultures. The highest activity was observed from the fraction 2 (eluted with 50% CH_2Cl_2 in hexane) which exhibited an activity of IC_{50} values at 1.28 and 1.14 μg mL^{-1} against W2 and D6 strains, respectively.

This fraction elaborated two major components viz naringenin-7-O-methyl ether (5) with the highest activity (2.75 μg mL^{-1} against W2 and 4.80 μg mL^{-1} g mL^{-1} against D6) and quercetin-4', 7-O-dimethylether (4) which had reduced activity at IC_{50} 13.98 and 10.48 μg mL^{-1} against W2 and D6 strains, respectively. Whereas, kaempferol-7-O-methylether (7) obtained from fraction 3 (eluted with 70% CH_2Cl_2 in hexane) had the 2nd highest activity (at IC_{50} 4.83 and 5.70 μg mL^{-1} against W2 and D6 strains, respectively), it shown an increased activity compared to the mother fraction at IC_{50} of 8.83 μg mL against W2 and 7.46 μg mL^{-1} against D6. Fraction 1 was the least potent at IC_{50} values of 10.42 μg mL^{-1} against W2 and 8.72 μg mL^{-1} against D6. This fraction yielded stigmasterol (2) as the major component. From the anti-plasmodial results (Fig. 1) obtained from the crude extracts, partitioned fractions and the pure isolates it is concluded that the crude extracts (surface compounds) shown potent *in vitro* activity against *Plasmodium falciparum* possibly due to the syngestic influence of the flavonoids components. Methanol extract was not very active due to the reduced quantities of these metabolites.

Isolation of the components of the fraction bioassayed was done using normal phase liquid CC on activated silica gel eluting with mixtures of hexane/CH_2Cl_2 on increasing polarity up to 5% methanol in CH_2Cl_2. Purification of the isolates was done using further prep-

TLC on silica gel G UV 254 nm and gel permeation CC on sephadex LH-20. A total of 7 compounds were realized; comprising 3 flavanones, two flavonols and 2 steroids. Structural assignments were made through extensive use of spectroscopic data correlated with literature data and experimental data (Table 1).

Substitution patterns were established through NMR, HMQC, HMBC, UV shift experiments and mass spectrometry fragmentation patterns. Compound 6 4', 5-Dihydroxy-6, 7-dimethoxyflavanone is a rare type of flavanone among the genus Gardenia due to its C-6 methoxylation. Assignment of the OCH_3 at C-6 was done on the assumption that C-6 would absorb at around δ130-132 ppm (Agrawal, 1989; Markham, 1982).

While oxygenation at C-8 would show a peak around ca. δ127 ppm[10]. Therefore a peak at δ131.04 ppm assigned for C-6 was corroborated by HMBC contours that exhibited ^3J correlation between protons δ3.80 ppm on δ60.78 ppm with δ131.04 ppm carbon.

The singlet peak for aromatic proton at 6.11 ppm shown ^2J correlation to ^{13}C peak at δ156. 53 ppm for C-8a and not to signal at δ162.00 for C-5. Furthermore, methoxylated C-6 would exhibit a downfield shift on the methoxyl group due to *diortho* substitution to oxygenated ring (Markham, 1982). The presence of a fragment ion at m z^{-1} 196 for ($C_9H_8O_6$) and m z^{-1} 120 for (C_8H_8O) resulting from a retro Diels-Alder ring cleavage of C-ring in the ionization chamber of MS therefore placed two methoxyl groups in A-ring and a hydroxyl group in B-ring.

However, compounds 3, 6 were isolated in small amounts and were not tested for anti-plasmodial activity.
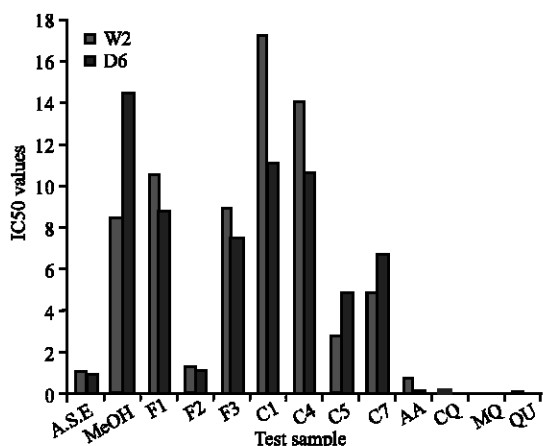
Fig. 2: The relative activities of the crude extracts, chromatographic fractions and pure isolates from *Gardenia ternifolia* against *Plasmodium falciparum*: ASE = Acetone Crude Extract, MeOH = MeOH extract, F1 = fraction1, F2 = fraction2, F3 = fraction3, C1 = compound 1, C4 = compound 4, C5 = compound 5, C7 = compound 7, AA = crude extract of *Artemisia annua*, CQ = chloroquine, MQ = mefloquine, QU = quinine

Table 2: LC_{50} values for larvicidal effects of extracts and compounds of *Gardenia ternifolia* against 2nd instar *Ae aegypti* larvae

Sample	LC_{50} µg mL^{-1} at 24 h	LC_{50} µg mL^{-1} at 48 h
MeOH extract	83.31±1.88	81.58±1.62
Acetone (SE)	32.01±1.64	28.02±1.24
Naringenin-4',7-O-dimethyl-ether (3)	NT	NT
Quercetin-4',7-O-dimethylether (4)	35.70±0.35	26.44±0.92
4',5-diOH-6,7-diOMe-flavanone (6)	NT	NT
Naringenin-7-O-methyl ether (5)	24.44±096	18.34±1.31
Kaempferol-7-O-methyl ether (7)	30.65±1.35	27.27±0.75

Triplicate trials; LC_{50} values are Mean±SD (n = 3) 95% CI, obtained by interpolation of probit values against log concentrations figures

This study lends support to the use of *Gardenia ternifolia* in the management of malaria. Naringenin-7-O-methyl ether was identified as the major constituent and most active anti-plasmodial principle *in vitro* within the aerial parts of the plant (Fig. 2).

Larval control in management of malaria or mosquito infestation through habitat modification or use of larval control agents that are target specific and environmental benign botanical alternative larvicide is an important strategy. Thus both acetone surface extract and the methanol extract were screened for larvicidal activity against 2nd instar *Aedes aegypti* larvae. The acetone surface extract was more active (LC_{50} 32.01±1.64 µg mL^{-1} at 24 h; 95% CI) compared to methanol extract (LC_{50} 83.31±1.88 µg mL at 24 h; 95%CI). Mortality of the larvae especially at higher concentrations of the samples was realized after 48 h of exposure and this associated largely with the surface exudates extracts. Among the pure isolates tested (Table 2) naringenin-7-O-methyl ether was the most active compound (LC_{50} 24.44±0.96 µg mL^{-1} at 24 h; 95%CI). At high concentrations the extracts and the pure compounds were lethal but at low concentration they inhibited growth. Where mortality was used as criterion for the effect of the extract on the larvae, it was found that at 100 µg mL extracts in the larval-rearing solution, survival of larvae did not exceed 48 h. At <50 µg mL^{-1} survival of larvae was high for up to >5 days but such larvae never attained adulthood. Growth-inhibition occurred in almost all the crude extracts and the pure isolates at low concentrations.

CONCLUSION

It is possible to note that maintaining unfed larvae for a long period of time may pose insignificant growth. Since some compounds may also have lead to poor growth due to antifeedant activity and not growth-regulatory effects per sec. However, more data may be required to determine the contribution of each of these compounds as growth regulators or as antifeedants.

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