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ORIGINAL PAPER

Biolarvicidal compound gymnemagenol isolated from leaf extract of miracle fruit plant, *Gymnema sylvestre* (Retz) Schult against malaria and filariasis vectors

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Abstract Owing to the fact that the application of synthetic larvicide has envenomed the surroundings as well as non-target organisms, natural products of plant origin with insecticidal properties have been tried as an indigenous method for the control of a variety of insect pests and vectors in the recent past. Insecticides of plant origin have been extensively used on agricultural pests and, to a very limited extent, against insect vectors of public health importance, which deserve careful and thorough screening. The use of plant extracts for insect control has several appealing features as these are generally more biodegradable, less hazardous and a rich storehouse of chemicals of diverse biological activities. Moreover, herbal sources give a lead for discovering new insecticides. Therefore, biologically active plant materials have attracted considerable interest in mosquito control study in recent times. The crude leaf extracts of *Gymnema sylvestre* (Retz) Schult (Asclepiadaceae) and purified gymnemagenol compound were studied against the early fourth-instar larvae of *Anopheles subpictus* Grassi and *Culex quinquefasciatus* Say (Diptera: Culicidae). In the present study, bioassay-guided fractionation of petroleum ether leaf extract of *G. sylvestre* led to the separation and identification of gymnemagenol as a potential new antiparasitic compound. Phytochemical analysis of *G. sylvestre* leaves revealed the presence of active constituents such as carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins. However, cardiac glycosides and phlobatannins are absent in the plant extracts. Quantitative analysis results suggested that saponin (5%) was present in a high concentration followed by tannins (1.0%). The 50 g powder was loaded on silica gel column and eluted with chloroform–methanol–water as eluents. From that, 16 mg pure saponin compound was isolated and analysed by thin layer chromatography using chloroform and methanol as the solvent systems. The structure of the purified triterpenoid fraction was established from infrared (IR), ultraviolet (UV), ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR and mass spectral data. The carbon skeleton of the compound was obtained by ^{13}C NMR spectroscopy. The chemical shift assignments obtained for gymnemagenol from ^{1}H NMR correspond to the molecular formula $C_{30}H_{50}O_4$. The compound was identified as 3β, 16β, 28, 29-tetrahydroxyolean-12-ene (gymnemagenol sapogenin). Parasite larvae were exposed to varying concentrations of purified compound gymnemagenol for 24 h. The results suggested that the larval mortality effects of the compound were 28%, 69%, 100% and 31%, 63%, 100% at 6, 12 and 24 h against *A. subpictus* and *C. quinquefasciatus*, respectively. In the present study, the per cent mortality were 100, 86, 67, 36, 21 and 100, 78, 59, 38 and 19 observed in the concentrations of 1,000, 500, 250, 125 and 62.75 ppm against the fourth-instar larvae of *A. subpitcus* and *C. quinquefasciatus*, respectively. The purified compound gymnemagenol was tested in concentrations of 80, 40, 20, 10 and 5 ppm, and the per cent mortality were 100, 72, 53, 30 and15 against *A. subpitcus* and 100, 89, 61,

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42 and 30 against *C. quinquefasciatus*, respectively. The larvicidal crude leaf extract of *G. sylvestre* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *A. subpictus* (LC_{50}=166.28 ppm, r^2=0.807) and against the larvae of *C. quinquefasciatus* (LC_{50}= 186.55 ppm, r^2=0.884), respectively. The maximum efficacy was observed in gymnemagenol compound with LC_{50} and r^2 values against the larvae of *A. subpictus* (22.99 ppm, 0.922) and against *C. quinquefasciatus* (15.92 ppm, 0.854), respectively. The control (distilled water) showed nil mortality in the concurrent assay.

Introduction

Humans have used plant parts, products and metabolites in vector control since early historical times. Plants are the chemical factories of nature, producing many chemicals, some of which have medicinal and insecticidal properties. Malaria is a parasitic disease from which more than 300 million people suffer yearly throughout the world. It is one of the main causes of infant and young child mortality (WHO 2005). About two million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by the World Health Organisation Southeast Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar et al. 2007). Lymphatic filariasis (LF) caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* is one of the major public health problems in Southeast Asia. The mosquito-borne disease accounts for the highest proportion of the population are at risk, or half of the worldwide cases (WHO 2001, 2008; Ottesen et al. 2008). It is estimated that there are 120 million cases of this disease worldwide, and over 90% of these infections are due to *W. bancrofti* (Ottesen 2006). LF is second only to malaria as the most important vector-borne disease in India. The disease is endemic in 18 States or Union territory, including the populous states of Uttar Pradesh and Bihar. Approximately 420 million people reside in endemic areas, and 48.11 million are infected. Bancroftian filariasis, caused by *W. bancrofti* and transmitted by the tropical house mosquito *Culex quinquefasciatus*, accounts for 95% of the total lymphatic filariasis cases in India (Ramaiah et al. 2000; Michael et al. 1996). The disease is found throughout sub-Saharan Africa, Southeast Asia, India, South America, and various Pacific islands and has been associated with significant morbidity in these regions (WHO 1992).

An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Lee et al. 2001). The chemicals derived from plants have been projected as weapons in future mosquito control programme as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent (Sukumar et al. 1991). Plants could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and are generally free from harmful effects. The use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the production cost and environmental pollution (Rawani et al. 2010). The search for herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control (Redwane et al. 2002). In the last decade, numerous studies have been conducted in different countries to prove such efficiency in a number of medicinal plants. Earlier researchers have reported the bioactivity of essential oils or on the effectiveness of plant extracts from various plants against the larvae of mosquitoes (Mehlhorn et al. 2005; Amer and Mehlhorn 2006a, b; Rahuman et al. 2009a, b).

Gymnema sylvestre (syn. *Periploca sylvestris* Retz) is a large woody climber plant, from the Asclepiadaceae family, and native to central and western India, tropical Africa and Australia. This plant has been used in Ayurvedic medicine for the treatment of diabetes in India for centuries. The plant is popularly known as 'gurmar' for its distinctive property of temporarily destroying the taste of sweetness (Reddy et al. 1998). The leishmanicidal activity of saponin, dasyscyphin C of *Eclipta prostrata*, and sapogenin, gymnemagenol isolated from *G. sylvestre* leaves tested against *Leishmania major*, *Leishmania aethiopica* and *Leishmania tropica promastigotes* was studied by the MTS assay (Khanna et al. 2009). The new saponins were identified as 21 beta-*O*-benzoylsitakisogenin 3-*O*-beta-D-glucopyranosyl(1→3)-beta-D-glucuronopyranoside (1), the potassium salt of longispinogenin 3-*O*-beta-D-glucopyranosyl (1→3)-beta-D-glucuronopyranoside (2) and the potassium salt of 29-hydroxylongispinogenin 3-*O*-beta-D-glucopyranosyl(1→3)-beta-D-glucuronopyranoside (3) and the aglycon of 3, gymnemagenol (3a), was characterized as 3 beta,16 beta,28, 29-tetrahydroxyolean-12-ene were isolated from an ethanol extract of the leaves of *G. sylvestre* (Ye et al. 2001). Subsequently, a number of oleanane-type triterpene saponins with different sapogenin structures such as gymnemagnenin, 23-hydroxylongispinogenin and gymnestrogenin, as well as certain dammarane derivatives, have been purified from plants growing in India (Yoshikawa et al. 1997). Daisy et al. (2009) have reported that an active compound dihydroxy gymnemic triacetate, which has been isolated from acetone extract of *G. sylvestre*, possessed hypoglycemic and hypo-

lipidemic activities in long-term treatment; hence, it could be used as a drug for treating diabetes. Two new oleanane-type triterpenoid saponins, gymnemoside-W1 and W2 (Zhu et al. 2008), the flavonoid triglycoside, kaempferol 3-*O*-beta-D-glucopyranosyl-(1→4)-alpha-L-rhamnopyranosyl-(1→6)-beta-D-galactopyranoside (Mukhopadhyay and Field 2006) and gymnemagenin (3 beta,16 beta,21 beta,22 alpha,23,28-hexahydroxy-olean-12-ene), the sapogenin of the anti-sweet principles (Liu et al. 1992), were isolated from *G. sylvestre*. Harborne (1996) reported that the leaves of the shrub *G. sylvestre* contain a complex of pentacyclic triterpenes–gymnemines or gymnemic acids reported as natural deterrents of insects and herbivorous animals. Kamaraj et al. (2010) reported that the adulticidal, repellent and larvicidal activities of crude hexane, ethyl acetate and methanol extracts of *Aristolochia indica*, *Cassia angustifolia*, *Diospyros melanoxylon*, *Dolichos biflorus*, *G. sylvestre*, *Justicia procumbens*, *Mimosa pudica* and *Zingiber zerumbet* were tested against adult and early fourth-instar larvae of *Culex gelidus* and *C. quinquefasciatus*. The methanol and dichloromethane extracts of leaves of *G. sylvestre* were tested against *Plasmodium falciparum* and for cytotoxic properties (Irungu et al. 2007).

The plant has been reported to possess antimicrobial (Sative et al. 2003) and ethno-veterinary medicinal properties (Kalidass et al. 2009). *G. sylvestre* leaves contain gymnemic acids, which are known to suppress transport of glucose from the intestine into the blood stream, and a small protein, gurmar, that can interact with receptors on the tongue to decrease the sensation of sweetness in many foods. The major bioactive constituents of *G. sylvestre* are a group of oleanane-type triterpenoid saponins known as 'gymnemic acids' (Hong-Min et al. 1992).

The crude acetone, hexane, ethyl acetate, methanol and petroleum ether extracts of the leaves of *Centella asiatica*, *Datura metal*, *Mukia scabrella* and *Toddalia asiatica* and extracts of whole plant of *Citrullus colocynthis* and *Sphaeranthus indicus* were assayed for their toxicity against the early fourth-instar larvae of *C. quinquefasciatus* (Rahuman et al. 2008a). The acetone crude extract of *Fagonia indica* and *Arachis hypogaea* (Chaubal et al. 2005), *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida* and *Trigonella foenum* (Harve and Kamath 2004) were tested against fourth-instar larvae of *Aedes aegypti* and *C. quinquefasciatus*. The methanol extracts of the dried root powder of *Rhinacanthus nasutus* (Rongsriyam et al. 2006) and *Chamaecyparis obtusa* (Jang et al. 2005) were tested against *A. aegypti* and *C. quinquefasciatus* larvae. The ethyl acetate leaf extract of *Acalypha indica* (Govindarajan et al. 2008); extract of fruit mesocarp of *Balanites aegyptiaca* (Wiesman and Chapagain 2006); the crude hexane extracts obtained from flower heads of *Spilanthes acmella*, *Spilanthes calva* and *Spilanthes paniculata* (Pandey et al. 2007); the methanol extracts of *Cryptomeria japonica* (Cheng et al. 2008); leaf extract of *Citrullus vulgaris* (Mullai et al. 2008); seed extracts of *Sterculia guttata* (Katade et al. 2006); the petroleum ether extract of *Solanum xanthocarpum* (Mohan et al. 2007); and leaf extracts of *Artemisia annua* and *Azadirachta indica* (Tonk et al. 2006) were tested against the larvae of *A. aegypti*, *Anopheles stephensi* and *C. quinquefasciatus*. Wood (2003) reported that some important phytochemical products such as pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, dlimonene, camphor and terpenes have been used as insecticides. Isoflavonoids from tubers of *Neorautanenia mitis* had a larvicidal effect against malaria- and filariasis-transmitting mosquitoes, *Anopheles gambiae* and *C. quinquefaciatus*, respectively (Joseph et al. 2004).

The benzene and methanol extracts of *Artemisia vulgaris* have a repellent activity against *A. aegypti* (Yit et al. 1985). *Zanthoxylum armatus*, *Zanthoxylum alatum* (Rutaceae), *A. indica* (Mailiaceae) and *Curcuma aromatica* (Zingiberaceae) possess repellent properties against mosquitoes (Das et al. 2000). The active compound octacosane from *Moschosma polystachyum* has repellent activity against the vector *C. quinquefasciatus* (Rajkumar and Jebanesan 2004). The essential oil of *Zingiber officinale* is a mosquito larvicidal and a repellent agent against the filarial vector *C. quinquefasciatus* (Pushpanathan et al. 2008). The methanolic leaf extract of *Cassia fistula* was tested for larvicidal and ovicidal activities against *C. quinquefasciatus* and *A. stephensi* (Govindarajan et al. 2008). The trypsin inhibitor isolated from *Cassia obtusifolia* showed significant inhibitory activity against trypsin-like proteases present in the larval midgut on *Pieris rapae* and could suppress the growth of larvae (Liao et al. 2007). The larvicidal activity of methanol extracts of *C. obtusifolia*, *Cassia tora* and *Vicia tetrasperma* was tested against early fourth-stage larvae of *A. aegypti* and *Culex pipiens* (Jang et al. 2002).

Several studies have been published concerning *G. sylvestre* activities which have shown that this species has antidiabetic, hypolipidaemic and antiatherosclerotic, insulinotropic, anti-inflammation, anticancer, cytotoxic, antioxidant, wound healing, leishmanicidal and antimicrobial activities (Ramachandran and Natarajan 2010). The aqueous extract of *G. sylvestre* leaves tested on various inflammatory models showed anti-inflammatory activity by significantly inhibiting carrageenan-induced rat paw oedema and peritoneal ascites in mice (Diwan et al. 1995). The plant is also reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic emetic, diuretic, stomachic, stimulant, antihelmenthics, laxative, cardiotonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, constipation, jaundice, haemorrhoids, renal–vesical calculi, cardiopathy, asthma, bronchitis, amenorrhoea, con-

junctivitis and leucoderma (Masayuki et al. 1997). The scientific knowledge of the plant is least explored. There is no report regarding the biological activity of this taxon (Ramachandran and Viswanathan 2009). Though there are many active principles, most of the studies are on the significant reduction of blood glucose; only a few reported on other biological activities.

The present study attempted to investigate the active compound and larvicidal efficacy of *G. sylvestre* leaf extract against *A. subpictus* and *C. quinquefasciatus* with the purpose of identifying effective indigenous bioproducts to control the vector of mosquito-borne diseases, particularly in cases where the vector's susceptibility to conventional synthetics is decreasing.

Materials and methods

Plant material

G. sylvestre (Asclepiadaceae; Fig. 1) leaves were selected on the basis of aromatic smell, bitter taste, resistance to damage by insect pests, and ethnopharmacological and ethnobotanical literature survey. The leaves were collected from Jawadi Hills (12°40′ N, 78°40′ E) Eastern Ghats, Tamil Nadu, India. The voucher specimen was prepared and deposited at the Herbarium section of the VIT University, Tamil Nadu, India. The leaves of *G. sylvestre* were washed with distilled water, shade-dried, powdered and stored in an air-tight container until further use.

Insect rearing

A. subpictus and *C. quinquefasciatus* larvae were collected from a rice field and stagnant water area of Melvisharam (12°56′23″ N, 79°14′23″ E) and identified in Zonal Entomological Research Centre, Vellore (12°55′48″ N, 79°7′48″ E), Tamil Nadu, to start the colony; larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method of Kamaraj et al. (2009).

Fig. 1 *G. sylvestre* (Asclepiadaceae)

Preparation of plant extracts

The leaves were dried for 7–14 days in the shade at environmental temperatures (27–37°C days time). The dried leaves (50 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with petroleum ether (100 mL, Fine), acetone (100 mL, Qualigens) and methanol (100 mL, Qualigens) in a Soxhlet apparatus (500 mL, boiling point range 60–80.8°C) for 8 h (Irungu and Mwangi 1995). The extract was concentrated under reduced pressure 22–26 mmHg at 45°C and the residue obtained stored at 4°C. During preliminary screening, the early fourth-instar larvae of *A. subpictus* and *C. quinquefasciatus* were used for our bioassay test. A total of 100 larvae were exposed in five replicates of 20 larvae each. Experiments were conducted for 24 h at room temperature (28±2°C). The experimental media, in which 100% mortality of larvae occurs alone, were selected for the isolation and purification of crude extracts. Among the crude extracts tested for larvicidal activity, the petroleum ether extract of *G. sylvestre* showed maximum activity and was selected for the purpose of isolation and purification of compounds by column chromatographic method.

Larvicidal bioassay

During preliminary screening with the laboratory trial, the larvae of *A. subpictus* and *C. quinquefasciatus* were collected from the stagnant water in and around the College Campus, Melvisharam, and identified in Zonal Entomological Research Centre, Vellore. One gram of crude extract was first dissolved in 100 mL of distilled water (stock solution). From the stock solution, 1,000 ppm was prepared with dechlorinated tap water. Early fourth-instar larvae were used for bioassay test. A total of 100 larvae were exposed in five replicates of 20 larvae each. Experiments were conducted for 24 h at room temperature (28±2°C). The control was set up with dechlorinated tap water. The experimental media, in which 100% mortality of larvae occurs alone, were selected for isolation and purification. The different fractions isolated were tested against the early fourth-instar larvae of mosquitoes by the procedure of WHO (1996), with some modifications, and as per the method of Rahuman et al. (2000). For the bioassay test, larvae were taken in five batches of 20 in 249 mL of water and 1.0 mL of plant extract concentration. From the stock solution, different concentrations ranging from 05 to 1,000 ppm were prepared. The numbers of dead larvae were counted after a 24-h exposure, and the percentage mortality was reported from the average of five replicates.

Extraction and purification of saponin

The powdered sample was defatted by petroleum ether for 3 h at 40°C. After filtering the petroleum ether, the sample was extracted with methanol for 3 h with mild heating. The methanol extract was concentrated and re-extracted with methanol and acetone (1:5, *v*/*v*; Yan et al. 1996).The precipitate obtained was dried under vacuum, which turns to a whitish amorphous powder after complete drying. It was loaded on Merck silica gel-60 (230–400 mesh) column and eluted with chloroform–methanol–water (70:30:10; Favel et al. 2005). The first fraction collected was air-dried at room temperature (28°C), and the residue obtained was treated as pure saponins of *G. sylvestre*. The purity of the saponin isolated was analysed by thin layer chromatography using chloroform and methanol (7:3) as the solvent system.

Structural elucidation

The purified saponins were subjected to structural elucidation by UV–visible spectroscopy (Techcomp), Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet −330), ^{1}H NMR spectroscopy (Jeol GSX-500), ^{13}C NMR spectroscopy (Jeol GSX-500) and mass spectroscopy (Finnigan MAT-8230). All the chemicals used for extraction and purification were of analytical grade, obtained from SRL, Mumbai, India.

Results

In the present study, parasite larvae were exposed to varying concentrations of crude leaf extract of *G. sylvestre* and purified compound gymnemagenol for 24 h. The results suggested that the mortality effects of purified compound gymnemagenol were 28%, 69%, 100% and 31%, 63%, 100% at 6, 12 and 24 h against *A. subpictus* and *C. quinquefasciatus*, respectively. The larvicidal crude leaf extract of *G. sylvestre* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *A. subpictus* (LC_{50}=166.28 ppm, r^2=0.807) and against the larvae of *C. quinquefasciatus* (LC_{50}=186.55 ppm, r^2=0.884), respectively (Table 1). The highest mortality was found in gymnemagenol compound at 80 ppm against the larvae of *A. subpictus* (LC_{50}=22.99 ppm, r^2=0.922) and against the larvae of *C. quinquefasciatus* (LC_{50}=15.92 ppm, r^2=0.854), respectively (Table 2). The control (distilled water) showed nil mortality in the concurrent assay. The chi-square value was significant at the $p \leq 0.05$ level.

Phytochemical analysis of *G. sylvestre* leaves revealed the presence of medicinally active constituents such as carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins. However, cardiac glycosides and phlobatannins are absent in the plant extracts. Quantitative analysis results suggest that saponins (5%) are present in a high concentration followed by tannins (1.0%, Table 3). The physicochemical properties of gymnemagenol are tabulated in Table 4. The fraction obtained from the silica gel column chromatography showed a single band in a thin layer chromatography (TLC) separation; the triterpenoid fraction was subjected to structural elucidation by spectroscopic studies (UV, FTIR, ^{1}H NMR, ^{13}C NMR and MS analysis). TLC was performed and a single spot containing gymnemagenol was confirmed (Fig 2). The UV spectrum of the fraction was recorded by scanning in the range of 200–450 nm using chloroform as solvent; the concentration is 0.5 mg/mL. The compound showed strong absorption band peak at 223, 237 and 274. FTIR spectrum was observed at

Table 1 Larvicidal activity of leaf petroleum ether extract of *G. sylvestre* against fourth-instar larvae of *A. subpictus* and C. *quinquefasciatus*

Species	Concentrations (ppm)	% Mortality[a] (ppm)±SD	LC_{50} (ppm)	LCL–UCL (ppm)	Slope	r^2
A. subpictus	1,000	100±0.00				
	500	86±0.981				
	250	67±0.532	166.28	141.26–195.72	36	0.807
	125	36±0.614				
	62.75	21±0.291				
C. quinquefasciatus	1,000	100±0.00				
	500	78±0.638				
	250	59±0.961	186.55	157.34–221.18	35	0.884
	125	38±0.743				
	62.75	19±0.357				

Control (distilled water), nil mortality. $P<0.05$, significant level

LC_{50} lethal concentration that kills 50% of the exposed larvae, *UCL* upper confidence limit, *LCL* lower confidence limit, r^2 regression coefficient

[a] Mean value of five replicates

Table 2 Larvicidal activity of purified compound gymnemagenol isolated from the leaves of *G. sylvestre* against fourth-instar larvae of *A. subpictus* and *C. quinquefasciatus*

Species	Concentrations (ppm)	% Mortality[a] (ppm)±SD	LC_{50} (ppm)	LCL–UCL (ppm)	Slope	r^2
A. subpictus	80	100±0.00				
	40	72±0.373				
	20	53±0.261	22.99	19.62–26.93	30	0.922
	10	30±0.822				
	05	15±0.351				
C. quinquefasciatus	80	100±0.00				
	40	89±0.272				
	20	61±0.421	15.92	12.66–20.02	42	0.854
	10	42±0.631				
	05	30±0.211				

Control (distilled water), nil mortality. $P<0.05$, significant level

LC_{50} lethal concentration that kills 50% of the exposed larvae, *UCL* upper confidence limit, *LCL* lower confidence limit, r^2 regression coefficient

[a] ean value of five replicates

3,445.41, 2,924.10, 1,635.38 and 1,457.48 cm^{-1}. The infrared spectra showed signals for two major functional groups identified as –OH (3,400–3,200 cm^{-1}), –CH (2,924 cm^{-1}), C=O (1,770 cm^{-1}) and C=C (1,635 cm^{-1}) corresponding to the chemical structure of saponins (Fig 3).

The chemical nature of the compound extracted from *G. sylvestre* was identified using the following spectral data. ^{1}H NMR (500 MHz in CDCl3),δ=5.182 (9H,m,H-18,H-19, H-24); 5.104(1H,t,H-6); 4.060(3H,dd,H-11,H-12); 3.505 (4H,S,H-16,H-17); 2.790(1H,t,H-1); 2.313 (3H,m,H-5,H-4); 2.291(2H,t,H-2); 2.084(1H,d,H-7); 2.029(4H,m,H-20,H-25, H-26,H-27); 1.619 (3H, m,H-14,H-15); 1.275 (10H,m,H-3, H-8,H-9,H-10,H-13); 1.235(3H,s,H-23); 0.876 (6H,m, H-21, H-22). ESI-MS *m/z* 474 $[M]^+$. The compound was identified as 3β,16β,28,29-tetrahydroxyolean-12-ene (gymnemagenol sapogenin). Mass spectroscopy data provided the molecular weight of the compound. The Finnigan MAT 8230 MS showed the $[M]^+$ ion at *m/z* 474 with the base peak at *m/z* 251. The proton NMR determines the structure of organic compound by measuring the magnetic moments of hydrogen atoms which are attached to different groups. The carbon skeleton of the compound is obtained by ^{13}C NMR spectroscopy. The chemical shift assignments obtained for gymnemagenol from ^{1}H NMR correspond to the molecular formula C_{30} H_{50} O_4 (Fig 4).

Table 3 Qualitative and quantitative analysis of the phytochemicals of crude extracts of *G. sylvestre* leaves

Phytochemicals	*G. sylvestre*	*G. sylvestre* (%)
Alkaloids	Present	0.5
Carbohydrates	Present	ND
Saponins	Present	05
Phytosterols	Present	ND
Phenols	Present	0.5
Flavonoids	Present	0.02
Terpenoids	Present	ND
Tannins	Present	1.0

ND not done

Discussion

Vector-borne diseases constitute the major cause of morbidity in most of the tropical and subtropical countries and have always been a challenge to the medical professionals struggling for the welfare of humanity. Plants are rich sources of bioactive compounds that can be used to develop

Table 4 Physicochemical properties of gymnemagenol

Colour	Colourless
Nature	Crystalline needle
Boiling point	1,295.87 K
Melting point	866.94 K
Critical temp.	1,065.47 K
Critical pres	12.65 bar
Critical vol.	1488.5 cm^3/mol
Gibbs energy	−185.38 kJ/mol
Log*P*	5.19
MR	135.92 cm^3/mol
Henry's law	6.46
Heat of form	−934.86 kJ/mol
CLog*P*	5.258
CMR	13.4594
Mol. formula	$C_{30}H_{50}O_4$

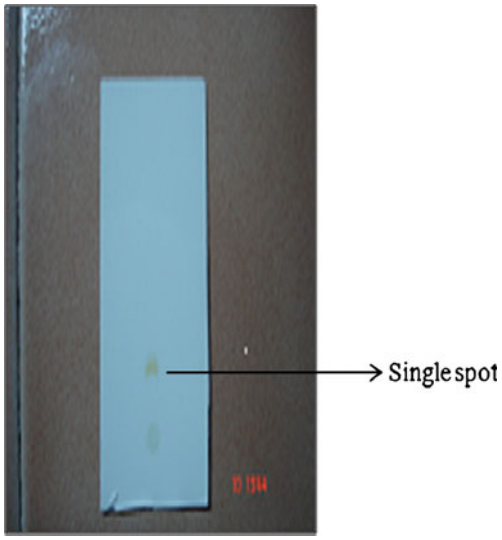

Fig. 2 Thin layer chromatograph showing a single spot identified as gymnemagenol

environmentally safe antiparasitic agents. Authors earlier reported that the crude and isolated compounds were tested against different mosquito species (Table 5). Several bioactive compounds such as dihydroxy gymnemic triacetate, gymnemic acid, deacyl gymnemic acid, gymnemagenin, gymnestrogenin, gymnemagenol, 23-hydroxylnogispinogenin, aglycon lupeol, β-amyrin, stigmasterol, pentriacontane, hentricontane, α- and β-chlorophyll, resin, tartaric acid, anthraquinone derivatives, alkaloids, betain, choline and trimethylamine have been isolated from *G. sylvestre* (Table 6). Several researchers have reported that *Gymnema* extracts (*G. sylvestre*, *Gymnema inodorum*, *Gymnema yunnanense*) may actually help to repair or regenerate the pancreas beta cells that are responsible for insulin secretion (Xie et al. 2003). In traditional systems of Indian medicine, *Gymnema* sp. is used for diabetes treatment, as a diuretic and a digestive stimulant (Chattopadhyay 1998). Recently, an active and novel compound, dihydroxy gymnemic triacetate, has been isolated from *G. sylvestre* acetone extract and its optimum dose has been determined (Daisy et al. 2009). Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine, thereby preventing sugar molecule absorption by the intestine, which results in low blood sugar level (Sahu et al. 1996).

Khanna and Kannabiran (2007) reported that the aqueous extracts of leaves of *G. sylvestre* caused 31%, 45% and 45% after 3 days with respect to 1%, 2% and 3% concentrations of the extract, respectively, against *C. quinquefasciatus* mosquito larvae; the larval mortality was below 50% in the 1%, 2% and 3% concentrations and showed 100% mortality during the second day with a 5% concentration. The compound gymnemagenol isolated from *G. sylvestre* showed 52% parasitic death at 1,000 μg/mL concentration against *L. major promastigote* (Khanna et al. 2009). Rahuman and Venkatesan (2008) reported that the petroleum ether extract of *C. colocynthis*, methanol extracts of *Coccinia indica*, *Cucumis sativus*, and *Momordica charantia* and acetone extract of *Trichosanthes anguina* against the larvae of *C. quinquefasciatus* showed LC_{50} values of 88.24, 377.69, 623.80, 207.61, and 842.34 ppm, respectively. The petroleum ether extracts of *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta* and *Euphorbia tirucalli* exhibited LC_{50} values of 11.34, 76.61, 113.40, 424.94 and 5.52 ppm, respectively, against *C. quinquefasciatus* (Rahuman et al. 2008b). The plant extracts exhibited a significant antifeedant activity after 24 and 48 h of exposure; however, the highest larval mortality was found in ethyl acetate extracts of leaves of *Anisomeles malabarica*, acetone

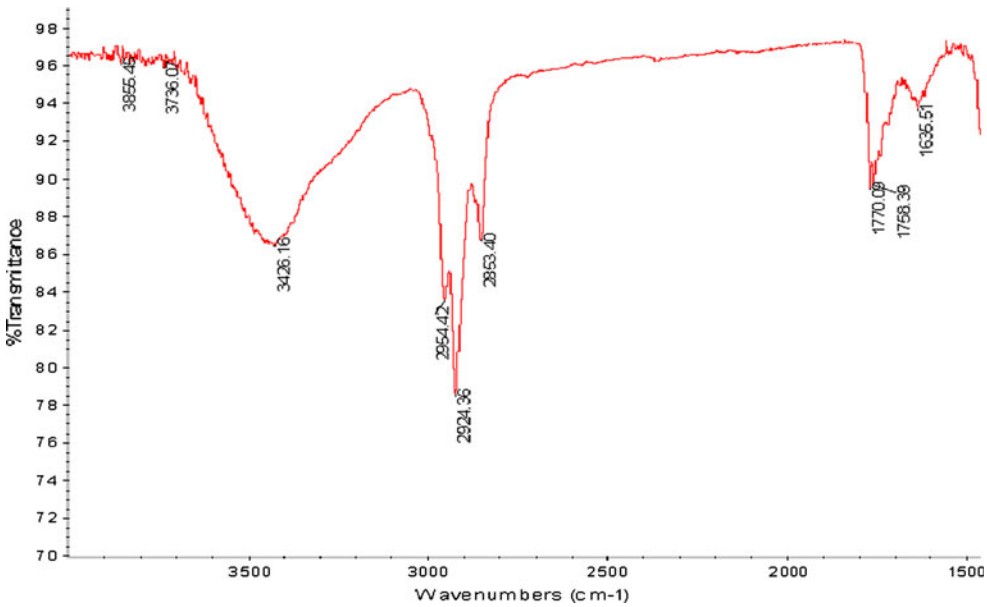

Fig. 3 FTIR spectrum of crude extract of *G. sylvestre* leaves

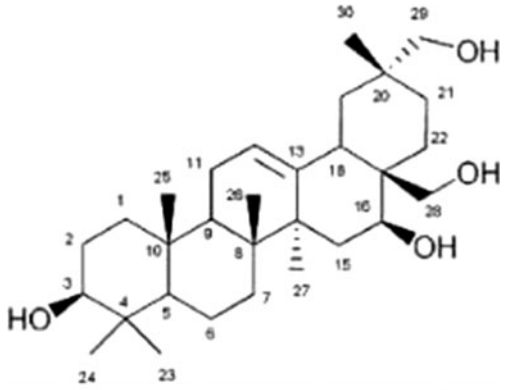

Fig. 4 Structure of gymnemagenol

extract of *E. prostrata*, methanol extract of *Andrographis lineata*, *Chrysanthemum indium* and *Sesbania grandiflora* after 24 h (LC_{50}=2.53, 2.82, 2.31, 2.56 and 2.08 mg/mL; LC_{90}=6.40, 8.06, 7.45, 6.98 and 6.20 mg/mL), respectively, and the hexane extract of *A. lineata* and *D. metal*; methanol extract of *Aristolochia bracteolata* and *E. prostrata* showed larval mortality after 48 h (LC_{50}=3.05, 2.11, 3.00 and 2.18 mg/mL; LC_{90}=9.06, 6.22, 8.23 and 5.77 mg/mL), respectively, against fourth-instar larvae of *A. subpictus* (Elango et al. 2011b). The highest adulticidal activity was observed in ethyl acetate extract of *A. lineata*, chloroform extract of *Andrographis paniculata*, acetone extract of *Cocculus hirsutus* and methanol extract of *Tagetes erecta* (LD_{50}=126.92, 95.82, 109.40 and 89.83 ppm; LD_{90}= 542.95, 720.82, 459.03 and 607.85 ppm). Effective adult emergence inhibition (EI) was found in leaf acetone extract of *Aegle marmelos*, ethyl acetate extract of *A. lineata*, and methanol extracts of *C. hirsutus* and *T. erecta* (EI_{50}=128.14, 79.39, 143.97 and 92.82 ppm; EI_{90}=713.53, 293.70, 682.72 and 582.59 ppm), respectively, against *A. subpictus* (Elango et al. 2011a). Santhoshkumar et al. (2011) have reported that the maximum efficacy was observed in crude leaf methanol, aqueous extracts of *Nelumbo nucifera* and synthesized silver nanoparticles against the larvae of *A. subpictus* (LC_{50}=8.89, 11.82 and 0.69 ppm; LC_{90}=28.65, 36.06 and 2.15 ppm). Bagavan et al. (2009a) have reported that the acetone, ethyl acetate extracts of *Annona squamosa*; methanol extract of *C. asiatica*; acetone extracts of *Gloriosa superba*; and ethyl acetate, hexane and methanol extracts of *Pergularia daemia* against *A. subpictus* (LC_{50}=17.48, 18.60, 26.62, 18.43, 34.06, 13.63 and 50.39 ppm), respectively. Zahir et al. (2009) investigated the leaf ethyl acetate extract of *Achyranthes aspera*, leaf chloroform extract of *A. malabarica*, flower methanol of *G. superba* and leaf methanol extract of *Ricinus communis* against the larvae of *A. subpictus* (LC_{50}= 48.83, 135.36, 106.77 and 102.71 ppm; LC_{90}=225.36, 527.24, 471.90 and 483.04 ppm), respectively. The highest larval mortality was found in leaf ethyl acetate of *A. marmelos*, *E. prostrata*, hexane, methanol of *A. paniculata* and *C. hirsutus* against the larvae of *A. subpictus* (LC_{50}= 167.00, 78.28, 67.24 142.83 ppm; LC_{90}=588.31, 360.75, 371.91 and 830.01 ppm), respectively (Elango et al. 2009). The highest mortality was found in leaf petroleum ether, flower methanol extracts of *C. auriculata*, flower methanol extracts of *L. aspera* and *R. nasutus*, leaf and seed methanol extracts of *Solanum torvum* and leaf hexane extract of *Vitex negundo* (LC_{50}=44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm; LC_{90}=187.31, 188.29, 233.18, 142.66, 151.60, 121.05 and 192.11 ppm, respectively (Kamaraj et al. 2009). The peel chloroform extract of *Camellia sinensis*, leaf ethyl acetate extracts of *Ocimum canum* and *Ocimum sanctum*, and leaf chloroform extract of *R. nasutus* showed LC_{50} values of 58.25, 88.15, 21.67 and 40.46 ppm; LC_{90} values of 298.31, 528.70, 98.34 and 267.20 ppm, respectively (Bagavan et al. 2009b).

Govindarajan et al. (2011) reported that the highest larval mortality was found in benzene extract of *Ervatamia coronaria* against the larvae of *C. quinquefasciatus*, with LC_{50} and LC_{90} values of 96.15 and 174.10 ppm. The most potent plant extract was the latex of *Calotropis procera* which killed 50% of the larval population at a concentration of 0.0062% (*v*/*v*) against the fourth-instar larvae of the lymphatic filariasis vector *C. quinquefasciatus* (Ali and El-Rabaa 2010). The highest larval mortality was found in the hexane extract of *Z. zerumbet*, ethyl acetate extract of *D. biflorus* and methanol extracts of *A. indica* against *C. quinquefasciatus* (LC_{50}=69.18, 34.76 and 25.60 ppm; LC_{90}=324.40, 172.78 and 105.52 ppm), respectively, after 24 h (Kamaraj et al. 2010). The corresponding LC_{50} value of leaf acetone, absolute alcohol, petroleum ether, chloroform/methanol (1:1, *v*/*v*), benzene and ethyl acetate extracts of *Solanum nigrum* were 72.91, 59.81, 54.11, 32.69, 27.95 and 17.04 ppm, respectively, after 24 h of exposure period against *C. quinquefasciatus* (Rawani et al. 2010). Changbunjong et al. (2010) reported that the ethanolic crude extract from *S. xanthocarpum* was investigated for its mosquito larvicidal activity; the LC_{50} against the larvae of *C. quinquefasciatus* was 573.20 mg/l while the LC_{90} was 1,066.93 mg/l. The alcoholic extract of *C. procera* (Asclepiadaceae) showed LC_{50} values of 109.71 and 387.93 mg/L for *A. stephensi* and *C. quinquefasciatus*, respectively, and the LC_{50} values were 13.06 and 86.47 mg/L, respectively, for latex of the plant (Shahi et al. 2010). Maurya et al. (2009) have reported the larvicidal potential of the various fruit wall extracts of *M. charantia*; the petroleum ether (LC_{50}=41.36 and 15.62 ppm) extract was found more effective than carbon tetrachloride (LC_{50}=80.61and 27.64 ppm) and methanol (LC_{50}=1,057.49 and 579.93 ppm) extract towards *C. quinquefasciatus* larvae after 24 and 48 h of exposure, respectively. The petroleum ether extracts of *Ageratum conyzoides* and *Argemone mexicana* have LC_{50} values of 425.60 and 267.90 ppm and

Table 5 Mosquito larvicidal activity of different plant isolated compounds

Plants/parts used	Larvicidal activity	Chemical compounds isolated	Lethal concentration	Reference
Chloroxylon swietenia/ leaves and stem	*Aedes aegypti* and *A. stephensi*	Geijerene	LC_{50}=43.4, 41.2; LC_{90}=76.8 and 73.5 μg/mL	Kiran et al. (2006)
		Pregeijerene	LC_{50}=28.3, 25.8; LC_{90}=45.6 and 38.6 μg/mL	
		Germacrene D	LC_{50}=63.6, 59.5; LC_{90}=100.7 and 96.4 μg/mL	
Asarum heterotropoides/root	*C. pipiens*, *A. aegypti* and *Ochlerotatus togoi*	Pellitorine	LC_{50}=2.08, 2.33 and 2.38 ppm	Perumalsamy et al. (2010)
Hymenaea courbaril/fruit peel	*A. aegypti*	Sesquiterpenes alpha-copaene	LC_{50}=14.8±0.4 μg/mL	Aguiar et al. (2010)
		Beta-selinene	LC_{50}=28.4±0.3 μg/mL	
Pistacia terebinthus L. subsp. *palaestina*/essential oil	*Culex pipiens*	α-Pinene	LC_{50}=59.2 ppm	Cetin et al. (2011)
Hypericum scabrum L/ essential oil	*C. pipiens*	α-Pinene	LC_{50}=82.2 ppm	
Vitex agnus castus L./ essential oil	*C. pipiens*	*trans*-β-Caryophyllene	LC_{50}=83.3 ppm	
Chrysanthemum coronarium L./essential oil	*C. pipiens*	Borneol	LC_{50}=311.2 ppm	
Guarea scabra/leaves	*A. aegypti*	*cis*-Caryophyllene and α-*trans*-bergamotene	LC_{50}=98.6 μg/mL	Magalhães et al. (2010)
G. convergens/branches	*A. aegypti*	Alpha-santalene	LC_{50}=145.1 μg/mL	
G. humaitensis/branches	*A. aegypti*	Caryophyllene epoxide and humulene epoxide II	LC_{50}=48.6 μg/mL	
G. silvatica/branches	*A. aegypti*	caryophyllene epoxide and spathulenol	LC_{50}=117.9 μg/mL	
Inula helenium	*A. aegypti*	Polyacetylenes	LC_{50}=1.07 μg/mL	Cantrell et al. (2010)
Croton heliotropiifolius	*A. aegypti*	Beta-caryophyllene	LC_{50}=159 ppm	Dória et al. (2010)
Zingiber officinale/Rhizome	*A. aegypti* and *C. quinquefasciatus*	4-Gingerol	LC_{50}=4.25 and 5.52 ppm	Rahuman et al. (2008a, b, c)
		Dihydrogingerdione	LC_{50}=18.20 and 27.24 ppm	
		6-Dehydrogingerdione	LC_{50}=9.80 and 7.66 ppm	
Phryma leptostachya var. asiatica/roots	*C. pipiens pallens*, *A. aegypti* and *Ocheratatos togoi*	Leptostachyol acetate	LC_{50}=0.41, 2.1 and 2.3 ppm	Park et al. (2005)
Melicope lunuankenda	*A. aegypti*	*p*-*O*-Geranylcoumaric acid	LC_{50}=20 μg/Ml^{-1}	Ramli et al. (2004)
Mansonia gagei	*A. aegypti*	Mansonone C	LC_{50}=50 ppm	Tiew et al. (2003)
Melicope subunifoliolata.	*A. aegypti*	Meliternatin	LC_{50}=1.45; LC_{90}=0.47 mg/mL	Ho et al. (2003)

140.15 and 137.70 ppm after a period of 24 and 48 h, respectively, and the methanol extract of *A. indica* have LC_{50} values of 21.95 and 11.30 ppm after 24 and 48 h of exposure, respectively (Sharma et al. 2009).

Batabyal et al. (2009) reported that the methanol extract of *A. indica* was observed the most potent with LC_{50} at 74.04 and 58.52 ppm and LC_{90} at 201.83 and 171.70 ppm compared with methanol extract of *M. charantia* with LC_{50} at 101.18 and 93.58 ppm and LC_{90} at 322.81 and 302.62 ppm and carbon tetrachloride extract of *R. communis* with LC_{50} at 144.11 and 92.44 ppm and LC_{90} at 432.42 and 352.89 ppm after 24 and 48 h, respectively. The carbon tetrachloride extract of *Aloe barbadensis* was the most effective, with LC_{50} values of 15.31 and 11.01 ppm after 24 and 48 h of exposure, respectively, followed by petroleum ether extract of *A. barbadensis*, carbon tetrachloride of

Table 6 Biological activity of *G. sylvestre* and its purified chemical compounds

Plant extract/isolated chemicals	Biological activity	References
Gymnema sylvestre crude extract	Antimicrobial activity, hypolipidemic activity and antioxidant activity	Sative et al. (2003); Patel et al. (2009); Daisy et al. (2009)
Gymnemic acids III, IV, V and VII	Antiviral activity, Antihyperglycemic activity	Sinsheimer et al. (1968); Yoshikawa et al. (1997)
Dihydrooxy gymnemic triacetate	Antihyperglycemic activity	Daisy et al. (2009)
Gymnemoside b	Antihyperglycemic activity	Yoshikawa et al. (1997)
21 beta-*O*-benzoylsitiakisogenin 3-*O*-beta-D-glucopyranosyl (1→3) beta-D-glucuronopyranoside	Antisweet activity	Ye et al. (2001)
Gymnemagenol	Mosquito larvicidal activity, antibacterial and antifungal, antiparasitic	Khanna and Kannabiran 2007, 2008; Khanna et al. 2009

Cannabis sativa, methanol extract of *A. barbadensis*, and methanol and petroleum ether of *C. sativa*, with LC_{50} values of 25.97, 88.51, 144.44, 160.78 and 294.42 ppm after 24 h and 16.60, 68.69, 108.38, 71.71 and 73.32 ppm after 48 h of post-treatment, respectively (Maurya et al. 2008). Rahuman et al. (2009a, b) have reported that the highest larval mortality was found in leaf acetone, chloroform, methanol and petroleum ether of *Canna indica* (LC_{50}=29.62, 59.18, 40.77 and 44.38 ppm; LC_{90}=148.55, 267.87, 165.00 and 171.91 ppm) against second-instar larvae (LC_{50}=121.88, 118.25, 69.76 and 56.31 ppm; LC_{90}=624.35, 573.93, 304.27 and 248.24 ppm) and against fourth-instar larvae and acetone, hot water, methanol and petroleum ether extracts of *Ipomoea carnea* (LC_{50}=61.17, 41.07, 41.82 and 39.32 ppm; LC_{90}=252.91, 142.67, 423.76 and 176.39 ppm) against second-instar larvae (LC_{50}=145.37, 58.00, 163.81 and 41.75 ppm; LC_{90}=573.30, 181.10, 627.38 and 162.63 ppm) and fourth-instar larvae of *C. quinquefasciatus*, respectively. Kamaraj et al. (2008) reported that the highest larval mortality was found in methanol extract of *O. canum* and *R. nasutus* and acetone extract of *O. sanctum* against *C. quinquefasciatus* (LC_{50}=44.54, 73.40 and 38.30 ppm), respectively.

Chowdhury et al. (2009) have reported that the chloroform and methanol extracts of mature leaves of *S. villosum* showed that the LC_{50} values for all instars were between 24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure period against *A. subpictus*. Essential oil of *Plectranthus amboinicus* was studied for its chemical composition—the chemical compound was carvacrol (28.65%); its larvicidal potential against the malarial vector mosquito *A. stephensi* recorded LC_{50} values of the oil of 33.54 (after 12 h) and 28.37 ppm (after 24 h). The LC_{90} values of the oil were 70.27 (after 12 h) and 59.38 ppm (after 24 h; Senthilkumar and Venkatesalu 2010). The larvicidal activity of saponin from *A. aspera* effective against *A. aegypti* and *C. quinquefasciatus* were 18.20 and 27.24 ppm, respectively (Bagavan et al. 2008). Wiesman and Chapagain (2006) reported that saponin extracted from the fruit of *B. aegyptiaca* showed 100% larvicidal activity against *A. aegypti* mosquito larvae.

The compounds tessmannic acid and methyltessmannoate were isolated from the $CHCl_3$ root bark extract of *Tessmannia densiflora* and exhibited activity against *A. gambiae* mosquito larvae with an LC_{50} value of 93 ppm after 24 h exposure. The compound tessmannic acid was nearly three times more active than its methyl ester, methyltessmannoate, with an LC_{50} value of 244 ppm after a 24-h exposure (Kihampa et al. 2009). Bioassay-guided fractionation and subtraction bioassays of the dichloromethane extract of the root barks of *Lantana viburnoides* sp. *viburnoides* contained active fractions of furanonaphthaquinones regioisomers (LC_{50}=5.48–5.70 ppm in 72 h) and the lantadene triterpenoid camaric acid (LC_{50}= 6.19 ppm in 72 h) as active principles, whilst the lupane triterpenoid betulinic acid (LC_{50}<10 ppm in 72 h) was obtained from the least active fraction against early fourth-instar larvae of *A. gambiae* (Innocent et al. 2008). Rahuman and Venkatesan (2008) have reported that the oleic and linoleic acids isolated from the whole plant petroleum ether extract of *C. colocynthis* showed a quite potent larvicidal activity against fourth-instar larvae of *A. aegypti* (LC_{50}= 8.80 and 18.20 ppm; LC_{90}=35.39 and 96.33 ppm), *A. stephensi* (LC_{50}=9.79 and 11.49 ppm; LC_{90}=37.42 and 47.35 ppm) and *C. quinquefasciatus* (LC_{50}=7.66 and 27.24; LC_{90}=30.71 and 70.38 ppm), respectively. The essential oil and the sesquiterpenes isolated from the leaves of *Chloroxylon swietenia* were screened for mosquitocidal activity by fumigant toxicity, and the essential oil had a pronounced mosquitocidal activity with an LD_{50} of 1.0, 1.2 and 1.7×10^{-3} mg cm^{-3}, respectively, against *A. gambiae*, *C. quinquefasciatus* and *A. aegypti*. The major sesquiterpene, germacrene D, performed better and proved to be a potential candidate with LD_{50} values of $1.8–2.8\times10^{-3}$ mg cm^{-3},

followed by pregeijerene and geijerene (Kiran and Devi 2007). The compounds (1, 2, 4–12) isolated from the petroleum ether extract of dried ground seeds of *Piper nigrum* showed insecticidal activity against the fourth-instar larvae of *A. aegypti* and *A. stephensi* (Rasheed et al. 2005). Cantrell et al. (2005) reported that the compounds spathulenol, intermedeol and callicarpenal isolated from the essential oil extracts from *Callicarpa americana* and *Callicarpa japonica* showed significant repellent activity against *A. aegypti* and *A. stephensi*. Three new limonoids comprising a vilasininoid 1 and two havanensinoids, 2 and 4, were isolated from the chloroform fractions of the methanol root barks of *Turraea wakefieldii* and *Turraea floribunda*, respectively. Compounds 1, 2 and 4 had LD_{50} values of 7.1, 4.0 and 3.6 ppm, respectively, and were more potent than azadirachtin, which had an LD_{50} value of 57.1 ppm when tested against the larvae of *A. gambiae* (Ndung'u et al. 2004). Six compounds that were isolated from the active tuber extracts of *N. mitis* fractions showed activity against *A. gambiae* adult mosquitoes at different concentrations (LC_{50} values: Pachyrrhizine (1)= 0.007 mg/mL, neotenone (2)=0.008 mg/mL, neorautanone (3)=0.009 mg/mL, neoduline (4)=0.005 mg/mL, 4-methoxyneoduline (5)=0.011 mg/mL and nepseudin (6)= 0.003 mg/mL; Joseph et al. 2004).

Two triterpenes, beta-amyrin and 12-oleanene 3beta,21-beta-diol, isolated as a mixture from the chloroform soluble fraction of an ethanol extract of *Duranta repens* were highly effective against the larvae of the mosquito *C. quinquefasciatus* (Nikkon et al. 2010). Repeated column chromatography of the EtOAc-soluble fraction of the aerial parts of *Dodonaea viscosa* led to the isolation of methyl dodovisate A (1), methyl dodovisate B (2), dodonic acid (3), hautriwaic acid (4), hautriwaic lactone (5), (+)-hardwickiic acid (6), 5alpha-hydroxy-1,2-dehydro-5,10-dihydroprintzianic acid methyl ester (7), strictic acid (8), dodonolide (9) and 5,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5′-(3-methylbut-2-enyl)-3,6-dimethoxyflavone (11) which were evaluated on larvicidal activity against the fourth-instar larvae of *Aedes albopictus* and *Culex pipens quinquefasciatus* (Niu et al. 2010). Bioassay-guided fractionation through flash chromatography led to the isolation of two larvicidal compounds, namely 9-oxoneoprocurcumenol and neoprocurcumenol, from rhizomes of *C. aromatica*; 9-oxoneoprocurcumenol exerted significant toxicity ($P<0.01$) on mosquito larvae with an LC_{50} value of 5.81 ppm and LC_{90} of 9.99 ppm compared to neoprocurcumenol with 13.69 and 23.92 ppm LC_{50} and LC_{90} values, respectively (Madhu et al. 2010). Bioassay-guided fractionation led to the isolation of 4-gingerol (1), (6)-dehydrogingerdione (2) and (6)-dihydrogingerdione (3) from petroleum ether extract of *Z. officinale*, which exhibited larvicidal activities against fourth-instar larvae of *A. aegypti* (LC_{50}=4.25, 9.80, 18.20 ppm) and *C. quinquefasciatus* (LC_{50}=5.52, 7.66, 27.24 ppm), respectively (Rahuman et al. 2008c). Tiwary et al. (2007) observed the larvicidal activity of linalool-rich essential oil of *Zanthoxylum armatum* against different mosquito species, viz. *C. quinquefasciatus* (LC_{50}=49 ppm), *A. aegypti* (LC_{50}=54 ppm) and *A. stephensi* (LC_{50}=58 ppm). Fraction A1 of ethanol extracts obtained from *S. guttata* seeds was found to be most promising; its LC_{50} was 21.552 and 35.520 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively (Katade et al. 2006).

Conclusion

This study demonstrated that the crude extract of *G. sylvestre* and gymnemagenol have excellent mosquito larvicidal activities against both *A. subpictus* and *C. quinquefasciatus*. Thus, gymnemagenol and the petroleum ether extract of *G. sylvestre* have potential to be developed as natural larvicidal agents. However, further investigation of the insecticidal action mode of gymnemagenol was needed. Moreover, these results could be useful in the research for selecting newer, more selective biodegradable and natural larvicidal compounds.

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