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Original article

The anti-leech potential of the solvent extract of Bornean neem leaves and ultra-high performance liquid chromatography-high-resolution mass spectrometry profiling

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ABSTRACT

Aquaculture plays a vital role in the Malaysian economy and food production however, sometimes the development is impeded by parasites. The parasitic leech *Zeylanicobdella arugamensis* (Hirudinea) infest the sea bass, snappers and groupers. They feed on the blood and cause secondary infection which could cause host death in a short time. In this study, the leaves of the neem plant (*Azadirachta indica*) (Meliaceae) have been selected as a natural product for the elimination of the parasitic leeches *Z. arugamensis* and to evaluate the phytochemical composition via ultra-high performance liquid chromatography–high-resolution mass spectrometry system (UHPLC-HRMS). The parasitic leeches were collected from Universiti Malaysia Sabah aquaculture facilities and challenged with various concentration of neem leaves methanol extract. The results revealed significant antileech activity of the methanol extract against *Z. arugamensis* with total mortality at the concentration of 25, 50 and 100 mg/ml in an average period of 35.81 ± 5.40, 14.18 ± 0.99 and 9.21 ± 1.51 min, respectively. The analysis of UHPLC-HRMS showed the existence of isorhamnetin, myricetin, myricetin 3-O-galactoside, trifolin, and quercetin (flavonoids), 2-(3,4-dihydroxyphenyl) acetamide, apocynin, p-coumaric acid, scopoletin, and, phloretin (phenolics), pulegone, and carvone (terpenoids). Thus, the research showed that methanol extract of neem leaves contained effective bioactive compounds with anti-leech effects. This study will be very helpful to fish farmers in Malaysia and other Southeast Asian countries to control the parasitic leeches in aquaculture using a natural product.

1. Introduction

Aquaculture is an important activity in Malaysia and a vital tool for the increment of export revenue, food production and employment (Othman et al., 2017). In 2018 the fisheries department of Sabah reported 159,773.11 tonnes of fishery products from aquaculture in Sabah, Malaysia (Department of Fisheries Sabah, 2020). Aquaculture is an important source of protein for over 30 million population of Malaysia. Unfortunately, the spread of diseases and parasitic infestation is impeding aquaculture developments (Venmathi Maran et al., 2009; Venmathi Maran et al., 2012).

In Malaysia, aquaculture farmers culture various types of marine fishes such as snappers, groupers and sea bass. The highly prevalent species of the groupers are hybrid grouper *Epinephelus fuscoguttatus* × *E. lanceolatus*, tiger grouper *E. fuscoguttatus*, orange-spotted grouper *E. coiodes* and Malabar grouper *E. malabricus*. The highly prevalent snappers are the yellow-streak snapper *Lutjanus lemniscatus*, mangrove snapper *L. argentimaculatus*, John's snapper *L. johnii* and red snapper *L. erythropterus* (Othman et al., 2017). The culture of groupers and snappers impeded greatly due to the infestation of some parasites (Venmathi Maran et al., 2009) in addition to marine parasitic leech *Zeylanicobdella arugamensis* de Silva, 1963 (Hirudinea, Piscicolidae) (Cruz-Lacierda et al., 2000; Ravi and Shariman Yahaya, 2017). They attached to the gills, eyes, and fins of the host, feed the blood by sucking and

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cause lesions and wounds on the body surface which leads to bacterial infection and ultimately result in host mortality within 3–4 days (Cruz-Lacierda et al., 2000). Farmers use formalin, pesticides and other chemicals for the disinfestation of the parasites (Shariff et al., 2000). Such chemicals are highly toxic to fish (Pitten et al., 2000). Thus, it is very important to develop a natural control agent to reduce the consumption of harmful chemicals. Plant-derived extracts can act as a biocontrol agent against leeches due to the presence of different metabolites (Bahmani and Rafieian-Kopaei, 2014).

The neem plant (*Azadirachta indica*) (Meliaceae) has been reported with insecticidal activity (Schmutterer, 1990), antiparasitic activity (Venmathi Maran et al., 2021) and antivenom activity against black-necked spitting cobra (*Naja nigricollis*) (Sani et al., 2020). The antihelminthic efficacy of the neem extract has been reported against the gastrointestinal parasite of animals (Jamra et al., 2015). Antibacterial potential of the aqueous extract of neem leaves has been reported against *Citrobacter freundii* gram-negative bacteria isolated from diseased fingerlings of *Oreochromis mossambicus* (Tilapia) (Thanigaivel et al., 2015). Malaysian neem plant leaves have been reported with antifungal activity against the fungi *Aspergillus niger* and *Candida albicans* (Arumugam et al., 2015). However, no research has been reported on the evaluation of the anti-leech potential of the methanol extract of the plant against *Z. arugamensis*. The study aimed to provide a natural control agent against the parasitic leech *Z. arugamensis* by the evaluation of the antiparasitic potential and phytochemical investigation of the methanol extract of the neem leaves.

2. Materials and methods

2.1. Plant collection and extraction

The leaves of the plant were obtained from Sabah, Malaysia. The dried leaves were powdered using a grinder to fine particles. Around 50 g of the leaves powder was dissolved in HPLC grade methanol (250 ml) in a flask and placed on the orbital shaker for 3 days at room temperature. The supernatant was filtered using Whatman No. 41 filter paper. The methanol residues were removed from the extract till it becomes thick via a vacuum rotary evaporator. Just before the sample was dried water (50 ml) was added and continue to run the rotary evaporator to eliminate all the methanol residue. Finally, the moistened samples were kept at −80 ^{0}C for 24 h and then lyophilized using a freeze drier.

2.2. Ultra-high performance liquid chromatography

Liquid chromatography (LC) was performed as described by Venmathi Maran et al. (2021) using Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Thermo Syncronis C18 column (2.1 mm × 100 mm × 1.7 μm); Thermo Fisher Scientific, Waltham, MA, USA). Briefly, column temperature was maintained at 55 °C and injection volume of 2 μL was applied. Solvent A (water topped with 0.1% HCOOH) and solvent B (acetonitrile topped with 0.1% HCOOH) were used as the mobile phase with a flow rate of 450 μL/min (Shah et al., 2020b; Venmathi Maran et al., 2021) The LC program was initiated at 0.5% of solvent B for 1 min, then from 0.5% to 99.5% solvent B in 15 min and maintained for 4 min. Then, the column was conditioned as initial and stabilized for 2 min before the next injection.

2.2.1. Data acquisition using high-resolution mass spectrometry system

Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) data were acquired on data-dependent acquisition mode using Thermo Scientific Q Exactive HF Orbitrap (Thermo Fisher Scientific, Waltham, MA, USA), which is equipped with heated electrospray ionization (HESI) probe. Both MS and MS/MS data were acquired with the setting as described by Venmathi Maran et al. (2021), and instrument calibration was performed before sample analysis.

2.2.2. Data analysis and compounds identification

Acquired data were processed and analysed using Thermo Scientific Compound Discoverer 3.0 software. Default settings were executed to perform background subtraction, retention time alignment, feature detection, elemental composition analysis, libraries matching, and fragment ion search (FISh) scoring (Venmathi Maran et al., 2021). Identification of compound was primarily based on the matching of MS/MS spectral against mzCloud and mzVault databases. Unmatched signals were reprocessed with ChemSpider database and backed with FISh scoring of above 50.

2.3. Antiparasitic bioassay

The parasitic leeches were collected from the infested hybrid groupers (*Epinephelus fuscoguttatus* × *E. lanceolatus*) (Fig. 1) from Universiti Malaysia Sabah. The selected adult parasitic leeches (0.8–1.2 cm long) were categorized into 5 groups (6 leeches per group) negative control (group 1), positive control (group 2, treated with 0.25% formalin solution), extract treatment (groups 3, 4 and 5 administered with 25, 50 and 100 mg/ml of the methanol extract of neem leaves). The selected concentrations were based on optimization. The behaviour of parasitic leeches was noticed keenly during the exposure (Fig. 2). Parasites inactivity and mortality were noted up to 120 mins. At the end of the experiment, the leeches were further observed for 720 mins (after every 60 min interval) (Shah et al., 2020c).

2.4. Physio-chemical parameters

Physio-chemical parameters including temperature, salinity, pH and dissolved oxygen in the solutions of control and extract treated groups were determined via YSI multiparameter.

2.5. Statistical analysis

The data were analyzed using IBM SPSS Statistics 25 Window package (IBM, Armonk, NY, US) and presented as mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was performed to determine the significant

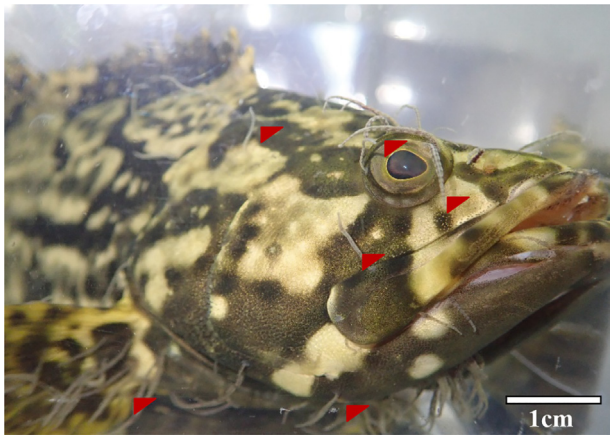


Fig. 1. Infestation of *Zeylanicobdella arugamensis* on hybrid grouper fish (*Epinephelus fuscoguttatus* × *E. lanceolatus*).

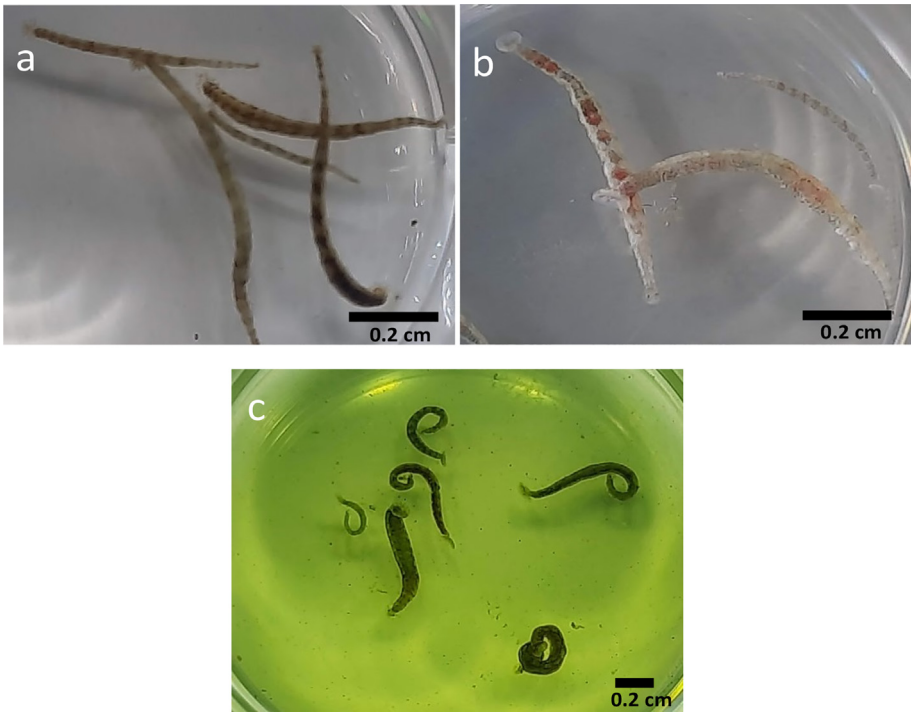


Fig. 2. Control and plant treated leeches. a = control, normal leech, treated with seawater only, b = leech treated with 0.25 % of formalin solution, c = leech treated with methanol extract of neem leaves.

differences between groups and means were considered as statistically significant if $p < 0.05$.

3. Results

3.1. Phytochemical profile of neem leaves methanol extract

The name of the metabolites noticed in the methanol extract of neem leaves via UHPLC-HRMS along with the class, retention time and formula are shown in Table 1 and the structures are indicated in Fig. 3. The data showed the presence of flavonoids (isorhamnetin, myricetin, myricetin-3-O-galactoside, trifolin, quercetin), aromatics (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 2,4-quinolinediol, indole-3-acrylic acid), phenolics (2-(3, 4-dihydroxyphenyl) acetamide, apocynin, phloretin, p-coumaric acid, scopoletin), and terpenoids (carvone, pulegone).

3.2. Antileech activity of neem leaves methanol extract

The administration of neem leaves extract resulted in the killing of leeches at lower (25 mg/ml), medium (50 mg/ml) and higher (100 mg/ml) concentration in an average period of 35.81 ± 5.40, 14.18 ± 0.99 and 9.21 ± 1.51 min. While an average time of 3.77 ± 0.25 min was taken by the formalin solution to kill the leeches. No mortality was noticed in the negative control group treated with seawater. Significance differences were noticed by multiple comparisons among the negative and positive control and extract-treated groups. The differences are highlighted by various symbols (Fig. 4). The mortality percentages of leeches are shown in Table 2. Total mortality (100 %) was noticed in leeches treated with neem leaves extract and formalin solution as compared to normal control group (no mortality).

3.3. The behaviour of leeches exposed to neem leaves methanol extract

At the concentration of 25 mg/ml of the extract, the leeches showed active swimming, but the movement reduced after 18 min and at the end of 30 min no movement was noticed. Similarly, active swimming of the leeches was noticed after exposure to 50 and 100 mg/ml of the extracts but after 10 and 2 min, respectively all the leeches become too weak. The leeches exposed to formalin also indicated active movement for 1 min before they stopped moving while the untreated leeches displayed a normal swimming pattern (Fig. 2).

3.4. Physio-chemical parameters

Water quality parameters are indicated in Table 3. Changes in the pH of the plant extract solution were noticed as compared to normal and positive control.

4. Discussion

The parasitic leech *Z. arugamensis* is well distributed throughout the Indian Ocean, affects a wide range of marine fish species (Cruz-Lacierda et al., 2000; Kua et al., 2014; Ravi and Shariman Yahaya, 2017). The leech resulted in the morality of the host in a very short period of time (Kua et al., 2014). Toxic and non-degradable drugs and chemicals are applied for the control of parasitic infestation (Forwood et al., 2013; Pitten et al., 2000). However, the chemicals could be hazardous to living organisms and the environment (Jayaraj et al., 2016; Pitten et al., 2000). Hence, to control the leech infestation, plants with potential bioactive compounds can be applied as a natural remedy (Kayser et al., 2003). Thus, the main reason for this study was to develop a natural and biodegradable control agent against parasitic leech by the application of the plant. Our study indicated that the methanol extract of neem leaves

Table 1
List of the 41 metabolites in the methanol extract of neem leaves identified using UHPLC-HRMS.

No.	Metabolites	Class	Retention Time	Formula	*m/z*	Adduct	Mass Error, ppm
[1]	Histidine	Amino acid	0.69	$C_6H_9N_3O_2$	156.07664	$[M + H]^+$	1.35
[2]	N3,N4-Dimethylarginine	Amino acid	0.85	$C_8H_{18}N_4O_2$	203.15031	$[M + H]^+$	0.45
[3]	Arginine	Amino acid	0.69	$C_6H_{14}N_4O_2$	175.11884	$[M + H]^+$	0.92
[4]	Valine	Amino acid	0.75	$C_5H_{11}NO_2$	118.08642	$[M + H]^+$	0.26
[5]	Leucine	Amino acid	0.90	$C_6H_{13}NO_2$	132.09036	$[M + H]^+$	1.07
[6]	1-Aminocyclohexanecarboxylic acid	Amino acid	1.20	$C_7H_{13}NO_2$	144.10190	$[M + H]^+$	0.35
[7]	Proline	Amino acid	0.94	$C_5H_9NO_2$	116.07070	$[M + H]^+$	1.04
[8]	Jasmone	Cyclic ketone	7.04	$C_{11}H_{16}O$	165.12721	$[M + H]^+$	0.97
[9]	2-Methylcyclohexan-1,3-dione	Cyclic ketone	1.50	$C_7H_{10}O_2$	127.07545	$[M + H]^+$	0.00
[10]	Decanamide	Fatty Acyl	8.09	$C_{10}H_{21}NO$	172.16940	$[M + H]^+$	0.88
[11]	4-oxododecanedioic acid	Fatty Acyl	5.17	$C_{12}H_{20}O_5$	245.13652	$[M + H]^+$	0.66
[12]	3-Hexenoic acid	Fatty Acyl	1.87	$C_6H_{10}O_2$	115.07545	$[M + H]^+$	1.23
[13]	Isorhamnetin	Flavonoid	5.54	$C_{16}H_{12}O_7$	317.06509	$[M + H]^+$	1.52
[14]	Myricetin	Flavonoid	4.91	$C_{15}H_{10}O_8$	319.04434	$[M + H]^+$	1.10
[15]	Myricetin 3-galactoside	Flavonoid	4.72	$C_{21}H_{20}O_{13}$	503.07938	$[M + Na]^+$	0.44
[16]	Trifolin	Flavonoid	5.41	$C_{21}H_{20}O_{11}$	471.08945	$[M + Na]^+$	1.16
[17]	Quercetin	Flavonoid	5.03	$C_{15}H_{10}O_7$	303.04953	$[M + H]^+$	1.79
[18]	6-Methyl-2-pyridinemethanol	Heterocyclic	1.08	C_7H_9NO	124.07578	$[M + H]^+$	0.73
[19]	Cytidine	Heterocyclic	0.71	$C_9H_{13}N_3O_5$	282.04807	$[M + K]^+$	1.07
[20]	Guvacoline	Heterocyclic	1.07	$C_7H_{11}NO_2$	142.08617	$[M + H]^+$	0.14
[21]	Glucosamine	Heterocyclic	0.61	$C_6H_{13}NO_5$	180.08640	$[M + H]^+$	0.78
[22]	Pyroglutamic Acid	Heterocyclic	0.83	$C_5H_7NO_3$	130.04984	$[M + H]^+$	0.46
[23]	β,β-Dimethyl-γ-methylene-γ-butyrolactone	Heterocyclic	1.61	$C_7H_{10}O_2$	127.07500	$[M + H]^+$	0.08
[24]	Pipecolic acid	Heterocyclic	1.05	$C_6H_{11}NO_2$	130.08623	$[M + H]^+$	0.85
[25]	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid	Heterocyclic aromatic	1.34	$C_{10}H_{11}NO_2$	178.08582	$[M + H]^+$	0.45
[26]	2,4-Quinolinediol	Heterocyclic aromatic	3.24	$C_9H_7NO_2$	162.05475	$[M + H]^+$	1.37
[27]	Indole-3-acrylic acid	Heterocyclic aromatic	1.94	$C_{11}H_9NO_2$	188.07060	$[M + H]^+$	0.59
[28]	Prolylleucine	Peptide	1.53	$C_{11}H_{20}N_2O_3$	229.15443	$[M + H]^+$	0.53
[29]	2-(3,4-dihydroxyphenyl)acetamide	Phenolid	0.93	$C_8H_9NO_3$	168.06551	$[M + H]^+$	0.54
[30]	Apocynin	Phenolic	2.93	$C_9H_{10}O_3$	167.06998	$[M + H]^+$	0.60
[31]	Phloretin	Phenolic	5.40	$C_{15}H_{14}O_5$	275.09100	$[M + H]^+$	1.50
[32]	p-Coumaric acid	Phenolic	3.21	$C_9H_8O_3$	165.05435	$[M + H]^+$	0.61
[33]	Scopoletin	Phenolic	4.35	$C_{10}H_8O_4$	193.04947	$[M + H]^+$	0.52
[34]	1-Methyladenine	Purine	0.83	$C_6H_7N_5$	150.07732	$[M + H]^+$	0.47
[35]	Guanine	Purine	0.79	$C_5H_5N_5O$	152.05652	$[M + H]^+$	1.26
[36]	2′-O-Methyladenosine	Purine	1.61	$C_{11}H_{15}N_5O_4$	282.11963	$[M + H]^+$	1.35
[37]	Adenine	Purine	0.80	$C_5H_5N_5$	136.06166	$[M + H]^+$	0.67
[38]	Carvone	Terpenoid	5.04	$C_{10}H_{14}O$	151.11153	$[M + H]^+$	0.87
[39]	Pulegone	Terpenoid	3.61	$C_{10}H_{16}O$	153.01779	$[M + H]^+$	0.72
[40]	Nicotinamide	Vitamin B3	0.89	$C_6H_6N_2O$	123.05534	$[M + H]^+$	0.66
[41]	Nicotinic acid	Vitamin B3	0.84	$C_6H_5NO_2$	124.03929	$[M + H]^+$	0.49

resulted in a total mortality of leeches at a concentration of 25 to 100 mg/ml in an average time period of 35.81 ± 5.40 to 9.21 ± 1.51 min, respectively. Some other plants with antiparasitic properties have also been reported recently. The methanol extract of fern *Nephrolepis biserrata* (Nephrolepidaceae) (100 mg/ml) resulted in a total mortality of *Z. arugamensis* in 4.8 min (Shah et al., 2020b), however the chromatographic fraction (2.50 mg/ml) took 1.9 mins (Shah et al., 2021). The methanol extract of neem leaves (100 mg/ml) took more time (9.21 min) for the total mortality of leeches compared to the methanol extract and fraction of *N. biserrata*. This might be due to the presence of various antiparasitic compounds of different nature in *N. biserrata* extract and fractions including ivalin, isovelleral, kaempferol 7,4′-dimethyl ether, piscidic acid, chlorogenic acid, phytosphingosine, pyrethrosin, warburganal, isodomedin and pheophorbide a (Shah et al., 2020b; Shah et al., 2021), however the neem leaves extract indicated completely different antiparasitic compounds (Table 1), discussed below in details. Similarly, the methanol extract of shrubby *Dillenia suffruticosa* (Dilleniaceae) (100 mg/ml) resulted in a total mortality of the parasitic leech in 14 min. (Shah et al., 2020c) and the chromatographic fractions (20 mg/ml) took 31 min. (Shah et al., 2020a). The methanol extract of the neem leaves indicated high antiparasitic efficacy in less time compared to the methanol extract of *D. suffruticosa*. All plants are available naturally in Sabah without any cost. Baikal Skullcap *Scutellaria baicalensis* (Lamiaceae) was tested against the marine parasitic leech *Piscicola geometra* (Hirudinea) infesting Cobia fish (*Rachycentron canadum*) (Rizky et al., 2018). The leeches were exposed to different serial dilutions (ranging from 1/50x to 1/1000x) of the extract for 96 h and mortality (100%) was recorded in an average time period of 8 h, 40 h, 48 h, 72 h, and 96 h (Rizky et al., 2018). The various serial dilutions (ranging from 1/50x to 1/1000x) of the extract of Noni *Morinda citrifolia* (Rubiaceae) was applied against *Piscicola geometra* for 96 h and 80% of leech mortality was reported (Rizky et al., 2018). The methanol extract of the ginger *Zingiber officinale* (Zingiberaceae) at a concentration of 32×10^4 ppm killed the freshwater leech *Limnatis nilotica* in 24 min (Forouzan et al., 2012). The above-mentioned reported plants evidently proved that the medicinal plants have anti-leech potential. In addition, compared to other plants the methanol extract of neem plant has shown an effective anti-leech activity in less than 10 min at higher concentration (see: Fig. 4) except *N. biserrata*.

The UHPLC-HRMS analysis of the methanol extract indicated the presence of compounds such as quercetin, carvone, phloretin, trifolin, jasmone and nicotinamide. The anti-leech activity of these compounds have not yet been reported against *Z. arugamensis* but they showed a various degree of potential effects against mammalian and plant parasites (Abdel-Rahman et al., 2013; Frank et al., 2002; Juan et al., 2013; Li et al., 2005; Mamani-matsuda et al., 2004; Mead and McNair, 2006; Sereno et al., 2005; Wuyts et al., 2006). In an animal study, a significant reduction in lengths of the gastrointestinal nematodes *Haemonchus contortus* has been reported in carvone treated sheep (Katiki et al., 2019). The application of carvone does not limit to mammalian parasites

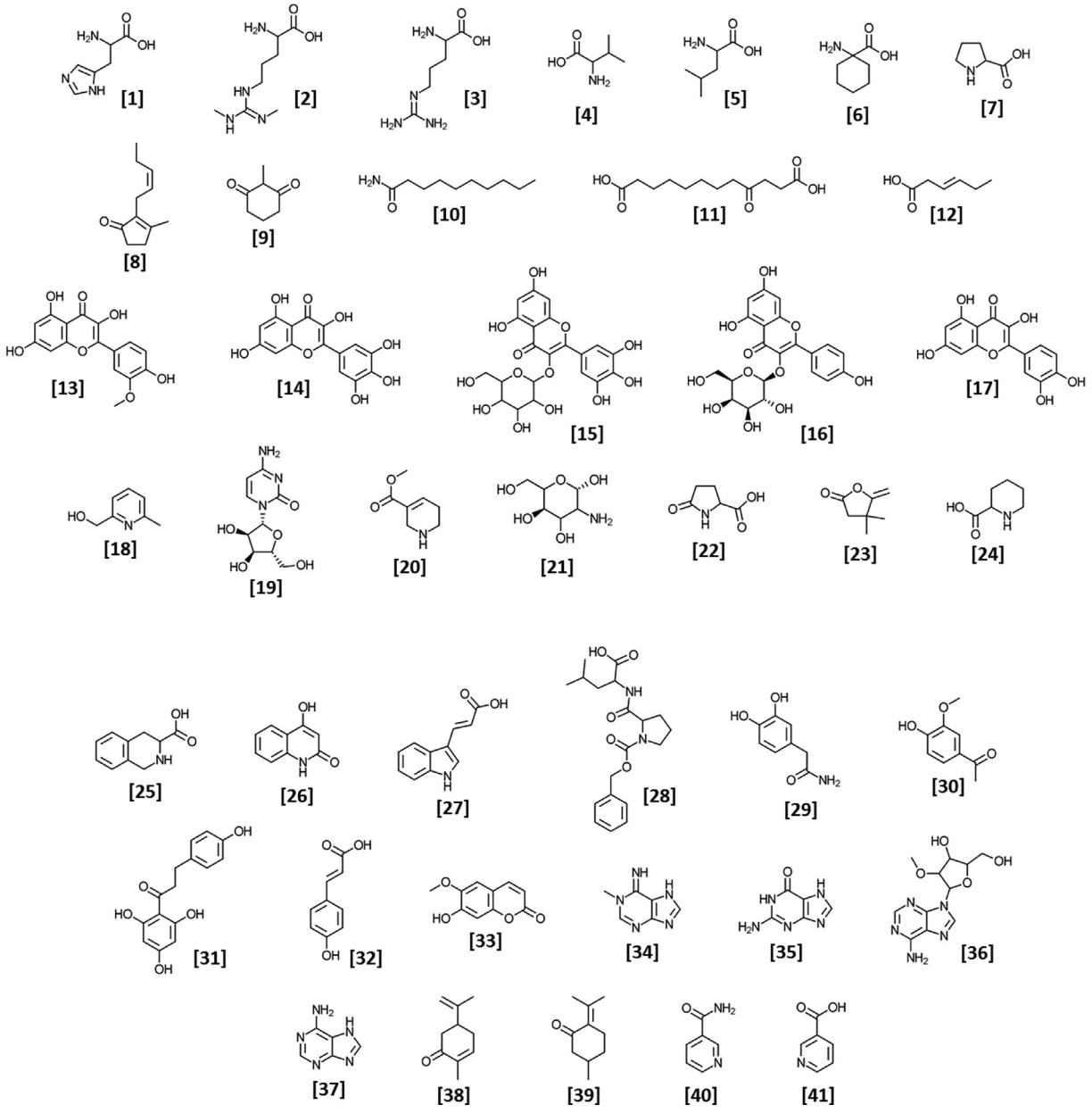
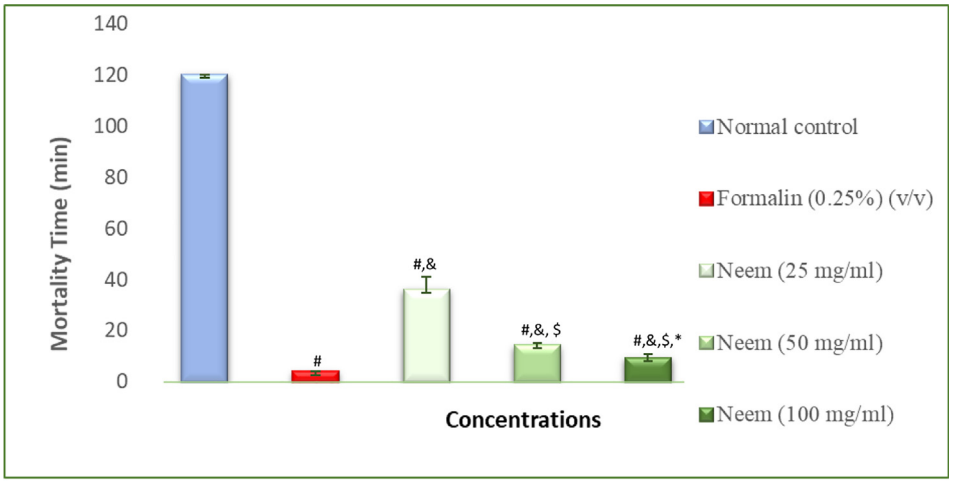


Fig. 3. Structure of the 41 identified metabolites from the methanol extract of neem leaves.

but plants as well. Aside from being effective in suppressing potato sprout, carvone has also been reported with antifungal properties against several plant parasitic fungi such as *Fusarium*, *Phoma*, and *Helminthosporium* (Hartmans et al., 1995; Oosterhaven et al., 1995). On the other hand, nicotinamide is also a well-documented antiparasitic compound against *Plasmodium falciparum* (Prusty et al., 2008), *Trypanosoma cruzi* (Soares et al., 2012), and *Leishmania* sp. (Sereno et al., 2005). Nicotinamide significantly deregulated the growth of *T. cruzi* by changing the conformation of sirtuin TcSir2, a NAD-dependent deacetylase which involved in the parasite cell cycle (Soares et al., 2012). Thus, we believe that the above-mentioned compounds in the methanol extract of neem leaves might contribute to the mortality of the parasitic leech. In the current study, the Bornean neem plant was easily accessible from nature without any cost. As mentioned above the crude extract of the plant possessed a mixture of vital antiparasitic chemical components. The cost of this naturally obtained chemical components mixture is very less as compared to the synthetically prepared chemical components.

5. Conclusion

Our data indicated that the methanol extract of neem leaves showed a significant anti-leech activity against the parasitic leech *Z. arugamensis* with a total mortality at various concentrations ranging from 25 to 100 mg/ml in average time period from 35.81 ± 5.40 to 9.21 ± 1.51 min, respectively, compared to untreated control. The ultra-high performance liquid chromatography–high–resolution mass spectrometry system revealed the presence of different bioactive compounds such as flavonoids, aromatics, phenolics, and terpenoids. Thus, it is indicated that the methanol extract of neem leaves act as an effective anti-leech agent against *Z. arugamensis* of cultured groupers due



Each value represents the mean±S.D of 6 parasitic leeches per group.
#Significance at $p<0.05$ compared with the control group.
&Significance at $p<0.05$ compared with the formalin treated group (0.25% v/v)
$Significance at $p<0.05$ compared with Neem leaves (25 mg/ml)
*Significance at $p<0.05$ compared with Neem leaves (50 mg/ml)

Fig. 4. Mortality time of parasitic leeches at different concentrations of methanol extract of neem leaves.

Table 2
Mortality percentage of parasitic leeches at different concentrations of methanol extract of neem leaves.

No	Group	Mortality of parasites in percentage (%)
1	Normal control	0
2	Positive control (Formalin 0.25%) (v/v)	100
3	Neem (25 mg/ml)	100
4	Neem (50 mg/ml	100
5	Neem (100 mg/ml)	100

Table 3
Water quality parameters of the solution for the treatment of parasitic leeches.

No	Water parameters		Concentrations			
	Groups	Normal control	Positive control (formalin 0.25%) (v/v)	Neem leaves (mg/ml)		
				(25)	(50)	(100)
1	Temperature (^{0}C)	24.6	25.3	24.8	24.7	25.1
2	pH	7.80	7.24	4.31	4.06	5.31
3	Salinity (ppt)	30.0	30.9	31	31.0	30.9
4	Dissolved oxygen (mg/l)	7.1	6.5	7.1	6.7	6.6

to the presence of vital bioactive compounds. Besides, our current sample indicated 100% mortality of *Z. arugamensis* in less time as compared to our previous sample *D. suffruticosa*. This research will be highly beneficial to fish farmers in Sabah, Malaysia to control the leeches in natural manners in aquaculture. However, further isolation, purification and characterization of the pure bioactive compounds with antiparasitic potential is required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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