










Chemical Composition and Pesticidal Activity of *Alpinia galanga* (L.) Willd. Essential Oils in Vietnam

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(Received May 13, 2021; Revised May 31, 2021; Accepted June 01, 2021)

Abstract: Mosquitoes are vectors of numerous pathogenic viruses and freshwater snails are intermediate hosts for several parasitic worms. There is a need for environmentally-benign botanical pesticides to augment or replace synthetic pesticides. In this research, essential oils from the leaves, stems, rhizomes, and roots of *Alpinia galanga*, an important Vietnamese medicinal plant, have been obtained by hydrodistillation and analyzed by gas chromatographic techniques. The essential oils were screened for mosquito larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus*, for molluscicidal activity against *Gyraulus convexiusculus* and *Pomacea canaliculata*, and for inhibition of acetylcholinesterase (AChE). The essential oils showed moderate to good pesticidal activities against these organisms. However, the oils also showed pronounced lethality to a non-target water bug, *Diplonychus rusticus*.

Keywords: Larvicidal; molluscicidal; *Aedes*; *Culex*; *Pomacea*; acetylcholinesterase. © 2021 ACG Publications. All rights reserved.

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1. Plant Source

Alpinia galanga (L.) Willd. (Zingiberaceae) naturally ranges from southern China and India, south through Indonesia, Malaysia, Myanmar, Thailand, and Vietnam [1]. The plant is an important herbal medicine throughout its range. The ethnomedicinal uses, pharmacological activities, and phytochemistry of this plant have been reviewed [2–4].

In this work, the leaves, stems, rhizomes, and roots of *A. galanga* were collected from Khanh Hoa Province, Vietnam, in February 2021, and identified by Do Ngoc Dai (See Table S1, in supporting information). Essential oils were obtained by hydrodistillation, analyzed by gas chromatographic techniques, and were screened for mosquito larvicidal, molluscicidal, and acetylcholinesterase inhibitory activities.

2. Previous Studies

Essential oils have reviewed for potential for use as eco-friendly mosquito larvicidal [5] or molluscicidal agents [6]. There have been numerous previous investigations of the essential oil of *A. galanga* (Table 1). To our knowledge, however, this is the first report on *A. galanga* essential oils from Vietnam and the first to examine the root essential oil, and to assess the mosquito larvicidal, molluscicidal, and AChE inhibitory activities of *A. galanga* essential oils. Extracts (hexane, dichloromethane, ethyl acetate, and methanol) of *A. galanga* rhizome have shown mosquito larvicidal activity and the active component was found to be (*E*)-cinnamic acid [7].

Table 1. Previous investigations on the essential oil of *Alpinia galanga*

Plant tissue	Collection location	Major components	Reference
Leaves	Calicut, Kerala, India	1,8-Cineole (28.3%), camphor (15.6%), β -pinene (5.0%)	[8]
Leaves	Bangalore, India	1,8-Cineole (34.4%), β -pinene (21.5%), camphor (7.8%), α -pinene (6.6%), camphene (5.0%)	[9]
Leaves	Hyderabad, India	1,8-Cineole (36.7%), β -pinene (23.5%), camphor (12.8%), α -pinene (6.3%), camphene (5.1%)	[9]
Leaves	Trivandrum, Kerala, India	α -Fenchyl acetate (20.7%), 1,8-cineole (6.5%)	[10]
Leaves	Trivandrum, Kerala, India	(<i>E</i>)- β -Caryophyllene (40.5%), caryophyllene oxide (7.2%)	[10]
Leaves	Pant, Nagar, Uttaranchal, India	1,8-Cineole (32.5%), β -pinene (22.7%), camphor (12.8%), α -pinene (5.6%)	[11]
Stems	Calicut, Kerala, India	1,8-Cineole (31.1%), camphor (11.1%), methyl cinnamate (7.4%)	[8]
Stems	Trivandrum, Kerala, India	Cubenol (28.4%), α -terpineol (5.5%), α -fenchol (5.2%), α -humulene (5.1%)	[10]
Rhizome	New Delhi, India	1,8-Cineole (57.0%), geranyl acetate (10.2%), (<i>E</i>)- β -caryophyllene (5.4%), 1-tricosanol (5.2%)	[12]
Rhizome	Nawinna, Sri Lanka	Zerumbone (44.8%), <i>p</i> -cymene (6.5%), camphene (6.4%), 1,8-cineole (6.3%)	[13]
Rhizome	Tenom, Sabah, Malaysia	1,8-Cineole (40.5%), β -bisabolene (8.4%)	[14]
Rhizome	Kuala Lumpur, Malaysia	1,8-Cineole (58.5%), (<i>E</i>)- β -farnesene (8.1%)	[15]
Rhizome	Purbalingga, Java, Indonesia	1,8-Cineole (45.2%), 4-vinylphenyl acetate (13.7%), α -farnesene (5.5%),	[16]
Rhizome	Calicut, Kerala, India	1,8-Cineole (28.4%), α -fenchyl acetate (18.4%), camphor (7.7%)	[8]
Rhizome	Bangalore, India	1,8-Cineole (33.6%), α -fenchyl acetate (12.7%), α -terpineol (9.3%), methyl cinnamate (5.3%), camphor (5.0%)	[9]

Pesticidal *Alpinia galanga* essential oils

Table 1 continued..

Rhizome	Hyderabad, India	1,8-Cineole (30.2%), camphor (14.0%), β -pinene (12.9%)	[9]
Rhizome	Trivandrum, Kerala, India	Carotol (26.7%), 1,8-cineole (10.8%), cubenol (9.5%), (<i>E</i>)- β -caryophyllene (5.8%)	[10]
Rhizome	Trivandrum, Kerala, India	1,8-Cineole (30.3%), α -terpineol (8.9%), α -fenchyl acetate (7.2%), β -pinene (6.5%), camphor (5.0%)	[10]
Rhizome	Kottayam, Kerala, India	1,8-Cineole (32.9%), α -terpineol (12.7%), germacrene D (6.1%)	[17]
Rhizome	Pant, Nagar, Uttaranchal, India	1,8-Cineole (39.4%), β -pinene (11.9%), α -terpineol (6.6%), α -pinene (5.6%), α -fenchyl acetate (5.6%), camphene (5.4%)	[11]
Rhizome	Imphal, Manipur, India	1,8-Cineole (53.4%), chavicol acetate (5.9%), β -sesquiphellandrene (5.0%)	[18]
Rhizome	Chiang Mai, Thailand	1,8-Cineole (46.2%), 4-allylphenyl acetate (9.4%), β -bisabolene (6.0%), β -pinene (5.2%)	[19]
Rhizome	Hulu Langat, Selangor, Malaysia	1,8-Cineole (61.9%), α -pinene (5.7%).	[20]
Rhizome	Thrissur, Kerala, India	1,8-Cineole (44.2-61.7%), (<i>Z</i>)- β -farnesene (7.0-14.6%), β -sesquiphellandrene (2.4-10.8%)	[21]

3. Present Study

The leaves, leaves and stems, rhizomes, and roots of *A. galanga* were hydrodistilled using a Clevenger apparatus to give the essential oils in 0.11%, 0.12%, 0.26%, and 0.13% yield, respectively.

The essential oils were analyzed by gas chromatography-mass spectrometry as previously described [22]. Compound identification was carried out by comparison of their mass spectral fragmentation patterns and retention indices with those recorded in the databases. The major components of the essential oils are summarized in Table 2.

Table 2. Major components of *Alpinia galanga* essential oils from Vietnam

RI _{calc}	RI _{db}	Compound	leaves	leaves & stems	rhizome	roots
933	933	α -Pinene	t	0.3	4.7	1.6
977	978	β -Pinene	t	0.1	0.6	9.0
1032	1032	1,8-Cineole	-	0.1	42.5	0.6
1339	1341	Indan-5-ol	-	-	20.2	0.4
1390	1392	<i>trans</i> - β -Elemene	5.6	8.6	0.8	0.6
1421	1424	(<i>E</i>)- β -Caryophyllene	15.8	20.0	1.9	5.7
1481	1480	Germacrene D	3.7	5.6	0.4	-
1505	1504	(<i>E,E</i>)- α -Farnesene	2.7	4.9	0.2	-
1519	1520	7- <i>epi</i> - α -Selinene	-	-	t	7.2
1582	1587	Caryophyllene oxide	7.4	5.3	0.1	20.3
1655	1655	α -Cadinol	0.6	0.8	0.4	2.7
1658	1660	Selin-11-en-4 α -ol	0.1	0.3	0.4	4.7
1834	1834	(<i>E,E</i>)-Farnesyl acetate	39.6	33.1	0.2	0.7
2105	2106	(<i>E</i>)-Phytol	4.5	2.5	-	-

RI_{calc} = Retention indices determined with reference to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Retention indices from the databases. t = trace (<0.05%). --- = not detected.

The essential oils from the leaves and stems, the rhizomes, and the roots of *A. galanga* were screened for mosquito larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* as previously described [23]. The essential oils were also screened for molluscicidal activity against *Gyraulus convexiusculus* and *Pomacea canaliculata*, and for insecticidal activity against the non-target water bug, *Diplonychus rusticus* as previously reported [24]. Lethality data were subjected to log-probit analysis to obtain LC₅₀ values, LC₉₀ values and 95% confidence limits using Minitab® version 19.2020.1 (Minitab, LLC, State College, PA, USA). The lethality data for the *A. galanga* essential oils are presented in Table 3. The essential oils were screened for inhibition of acetylcholinesterase (AChE) as previously described [24]. The essential oils from the leaves and stems and the roots showed moderate AChE inhibition with IC₅₀ values of 24.7 µg/mL and 91.7 µg/mL, respectively. The rhizome essential oil showed excellent AChE inhibition (IC₅₀ 5.32 µg/mL), comparable to the positive control galantamine (IC₅₀ 1.80 µg/mL).

Table 3. Mosquito larvicidal activity, molluscicidal activity, and insecticidal activity of *Alpinia galanga* essential oils from Vietnam.^a

Organism	LC ₅₀ (95% confidence levels)	LC ₉₀ (95% confidence levels)	χ ²	p
leaves & stems				
<i>Aedes aegypti</i> (24 h)	53.61 (49.29-59.04)	77.89 (70.55-88.75)	5.481	0.065
<i>Aedes aegypti</i> (48 h)	38.65 (35.13-42.56)	72.06 (63.06-86.22)	8.178	0.017
<i>Culex quinquefasciatus</i> (24 h)	41.56 (37.15-46.71)	96.18 (81.08-120.76)	5.901	0.117
<i>Culex quinquefasciatus</i> (48 h)	22.55 (19.93-25.48)	60.21 (50.56-75.43)	4.963	0.175
<i>Gyraulus convexiusculus</i>	22.87 (20.91-25.28)	35.34 (31.93-40.19)	3.206	0.524
<i>Pomacea canaliculata</i>	14.02 (12.58-15.64)	25.15 (22.63-28.66)	0.5454	0.969
<i>Diplonychus rusticus</i> (24 h)	9.123 (8.443-9.858)	13.06 (12.09-14.38)	3.279	0.512
<i>Diplonychus rusticus</i> (48 h)	9.062 (8.379-9.800)	13.05 (12.07-14.38)	3.733	0.443
rhizome				
<i>Aedes aegypti</i> (24 h)	47.18 (44.51-49.91)	59.78 (55.99-66.23)	0.0198	1.000
<i>Aedes aegypti</i> (48 h)	36.81 (34.30-39.51)	50.72 (47.28-55.22)	2.308	0.679
<i>Culex quinquefasciatus</i> (24 h)	21.68 (12.59-28.71)	78.08 (67.06-95.82)	0.0283	0.986
<i>Culex quinquefasciatus</i> (48 h)	17.01 (9.79-22.20)	54.62 (46.88-67.80)	0.2803	0.869
<i>Gyraulus convexiusculus</i>	46.86 (43.57-50.87)	65.30 (59.82-73.48)	1.626	0.653
<i>Pomacea canaliculata</i>	17.15 (15.89-18.56)	24.29 (22.48-26.67)	3.155	0.532
<i>Diplonychus rusticus</i> (24 h)	36.65 (33.72-39.53)	52.77 (49.22-57.32)	18.29	0.000
<i>Diplonychus rusticus</i> (48 h)	35.04 (32.09-37.91)	51.47 (47.96-55.95)	19.16	0.000
roots				
<i>Aedes aegypti</i> (24 h)	25.57 (23.50-28.19)	37.47 (33.87-42.87)	4.487	0.213
<i>Aedes aegypti</i> (48 h) ^b	25.57 (23.50-28.19)	37.47 (33.87-42.87)	4.487	0.213
<i>Culex quinquefasciatus</i> (24 h)	38.52 (34.80-43.00)	66.16 (59.28-75.95)	3.973	0.264
<i>Culex quinquefasciatus</i> (48 h)	26.07 (22.56-29.87)	55.97 (49.35-65.74)	5.589	0.133
<i>Gyraulus convexiusculus</i>	9.975 (9.037-10.988)	18.50 (16.32-21.77)	6.712	0.152
<i>Pomacea canaliculata</i>		not tested ^c		
<i>Diplonychus rusticus</i> (24 h)		not tested ^c		
<i>Diplonychus rusticus</i> (48 h)		not tested ^c		

^a The leaf essential oil was not screened due to insufficient material. ^b There were no additional deaths from 24 to 48 h. ^c Not tested due to insufficient essential oil.

The essential oils of *A. galanga* showed good mosquito larvicidal activities with 24-h LC₅₀ values less than 100 µg/mL and moderate molluscicidal activity (LC₉₀ ranging from 18 µg/mL to 65

Pesticidal *Alpinia galanga* essential oils

µg/mL). Unfortunately, the essential oils also showed comparable lethality to the non-target insect, *Diplonychus rusticus*. Thus, *A. galanga* essential oils are not likely to be viable botanical pest-control agents in aquatic systems.

Acknowledgments

This research was funded by University of Khanh Hoa, grant number KHTN-20.02. P.S. and W.N.S. participated in this work as part of the activities of the Aromatic Plant Research Center (APRC, <https://aromaticplant.org/>).

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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