ISSN 2598 0580

Bioscientia Medicina Volume 2, Issue 2, Page No: 31-41
Available online at: www.bioscmed.com
Bio Sc Med 2(2) :31-41

The Efficacy of Red Ginger Fraction (*Zingiber officinale* Roscoe *var. rubrum*) as Insecticidal *Aedes aegypti*

KM Yahya Syukur[1], Chairil Anwar[2#], Dalilah[2], Salni[3], Novrikasari[4]

[1]Postgraduate Student of Biomedical Science, Faculty of Medicine, Universitas Sriwijaya
[2]Faculty of Medicine, Universitas Sriwijaya
[3]Faculty of Science, Universitas Sriwijaya
[4]Faculty of Public Health, Universitas Sriwijaya

#Correspondence: chairil53@yahoo.co.id

Received : February 9th 2018

Accepted : March 20th 2018

Abstract

Background: *Aedes aegypti* is a vector of Dengue Haemorrhagic Fever. The use of synthetic chemical insecticide is the most common way to control *Ae. aegypti*. However, since it can cause resistency if it is used in a wide scale with high frequency and continously, it requires an alternative way. Red ginger (*Zingiber officinale* Roscoe *var. rubrum*) is one of natural insecticide which is safe to be used to control *Ae. aegypti*. This research aims was to determine the activity of active fraction of red ginger to mortality of *Ae. aegypti*.
Methods: It was a quasi-experiment research with post test only control design. Twenty *Ae. aegypti* Liverpool strain were the sample of this research with 5 concentration for each and 4 times repetition.
Results: The result showed that concentration 0.5%, 1.0%, 1.5%, 2.0% and 2.5% causing mortality percentage of 32.5%, 33.8%, 51.3%, 58.8% and 65.0% respectively. One way Anova test showed *p-value* < α (0.00 < 0.05).
Conclusion: It was concluded that there was a significant difference of various concentration of active fraction n-heksan of red ginger extract to the mortality of *Ae. aegypti*. The lethal concentration (LC_{50}) was 2.409%.

Key words: Toxicity, extract, fraction, *Aedes aegypti*, Insecticidal

Introduction

Dengue Hemorrhagic Fever (DHF) is an acute disease caused by dengue virus that goes into human blood circulation through *Aedes aegypti* mosquito bites. *Ae. aegypti* is the most common vector of DHF diseases. Mosquitoes carry dengue virus after sucking the blood of people who have been infected with the virus. After the incubation period of the virus in the mosquito's body for 8-10 days, the infected mosquito can transmit the dengue virus to the healthy human being it bites. Before 1970, only 9 countries had experienced severe dengue endemic. The disease is now endemic in more than 100 countries in the WHO region. America, Southeast Asia and the Western Pacific's worst affected areas exceed 1.2 million in 2008 and more than 3 million in 2013.[1]

Based on WHO data from 2004 to 2009, Indonesia is the country with the highest number of DHF patients in Southeast Asia. In Indonesia, since first discovered in 1860 by Walker in Ujung Pandang, dengue disease began to be known in Indonesia since 1968 in Surabaya with the number of patients with 58 people or 41.3% of deaths. The peak of dengue fever cases in Indonesia occurred in 2009 with the prevalence of 68.22 patients per 100,000 populations. And after that the number of dengue cases continues to increase along with the increasingly widespread endemic areas of DHF. In the last three years (2008-2010) the average number of cases was reported as many as 150,822 cases with an average death of 1,321 deaths. Incident rate in 2010 has reached 65.62/100,000 population with case fatality rate equal to 0.87%.[2]

In Indonesia, in 2013 the number of reported DHF is 112,511 cases with 871 deaths. An increase in the number of cases in 2013 compared to 2012 which amounted to 90,245 cases with IR 37.27. In 2013, Jambi Province, Bangka Belitung Islands and East Nusa Tenggara have a CFR > 2%.[1]

In South Sumatera, the number of DHF cases in 2014 amounts to 1,506 cases (IR of 19/100,000 population) with mortality of 4 deaths (CFR 0.27%). The most cases of dengue are in Palembang 622 cases, Prabumulih 226 cases, Banyuasin 136 cases, Muara Enim as many as 91 cases, and Ogan Ilir as many as 87 cases. By 2015, the number of DHF cases in South Sumatera Province is 3,401 cases with IR as much as 42.4 per100,000 populations.[3]

Palembang City as a city located in South Sumatera Province which has endemic category for DHF disease. In 2010 the number of DHF patients in Palembang as many as 675 people then increased in 2011 as many as 723 people. The number of DHF patients increased again in 2012 as many as 883 people. While in 2013, the number of DHF patients as many as 438 people, and in 2014 as many as 603 people with DHF.[4]

The absence of drugs and DHF vaccines, the government and the community have tried to reduce the increase of DHF cases by using spraying, mosquito nets, 3M movement (draining, burying and closing) and abate sowing to prevent the development of larvae from vector spreader of dengue virus. Control of *Ae. aegypti* mosquitoes as a vector of DHF has been done by reducing mosquito population or by deciding its life cycle. One way of using synthetic chemical insecticides such as DDT, ethylheksanadiol, temephos, and various other synthetics compounds. The use of synthetic insecticides for mosquito control can be useful when used in appropriate circumstances. But when used on a broad scale, continuously in the long run and with high frequency it can lead to decreased susceptibility (resistance).[5]

Efforts to reduce the use of synthetic chemical insecticides are very wise to do, when optimizing the use of plants that have the ability as a biological insecticide. In Indonesia there are many species of bio-insecticide-producing plants that are toxic to insects and there are approximately 2,400 species of plants.[6-7] One of the biological insecticides that have been studied to eradicate *Ae. aegypti* mosquito larvae is the red ginger rhizome (*Z. officinale* Roscoe *var. rubrum*).[8]

Red ginger (*Z. officinale* Roscoe *var. rubrum*) has the potential as a vegetable pesticide because it contains oleoresin compounds that give spicy flavor to ginger, as well as essential oil compounds containing many components, such as zingiberene, zingiberol and kaemferol.[9] The main components of essential oils of ginger that cause smell are zingiberene and zingiberol. While the main component contained in oleoresin compound is gingerol which has high antioxidant activity.[10]

Zingiberene acts as a receptor that can activate anti-feeding signals on insect central nerves, causing insects to not smell and recognize the presence of adjacent food. Inhibition of olfactory organs and damage to the gastrointestinal tract will decrease the activity of eating larvae causing the larva to become weak and die slowly.[8] Zinc ketone compound, which is a derivative of the zingiberene compound capable of decreasing the activity of insect feeding. In addition, red ginger also contains antioxidant compounds that can help neutralize the damaging effects caused by free radicals in the body. Zingiberol is sesquiterpen alcohol (C15H26O) which causes a distinctive aroma in ginger oil.[10] Kaemferol (flavanoid) acts as a strong respiratory inhibitor for insects and is able to block the olfactory organs in insect bodies, so the insect's respiratory system is disturbed.[11]

Some related research on ginger extract (*Z. officinale* Roscoe *var. rubrum*) was once performed as a larvicidal. The active compound in red ginger will react with the larval cell membrane and damage it causing lysis and disrupt the permeability of the plasma membrane. This resulted in leakage of the cytoplasmic membrane due to the breakdown of phospholipid molecules due to H^+ ions from the ginger compound one of them gingerol. Due to the breakdown of the cytoplasmic membrane will result in toxic compounds in red ginger liberally penetrate the larvae body and cause physiological disturbance in the body of the larvae.

The disruption of the larval physiological system results in disruption of the respiratory system, disrupting hormonal work and destroying the digestive system. In addition, the content of kaemferol present in ginger is able to enter into the larval breathing system and damage the mitochondrial work. Mitochondrial damage inhibits the electron transport process so that the energy metabolism process is disrupted and the decrease of adenosine triphospate (ATP) formation. A decrease in ATP production in the larvae causes the body of the larvae weak.[8] Thus, ginger extract (*Z. officinale* Roscoe *var. rubrum*) is thought to be capable of as a natural biolarvacida in insects. However, research on the effect of bioactive extracts of ginger extract (*Z. officinale* Roscoe *var. rubrum*) against *Ae. aegypti* mosquitoes has never been done.

Based on the above description it is necessary to conduct an active fraction separation study of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) essential oils, oleoresin (gingerol) and flavonoids in various concentrations of *Ae. aegypti* adult mosquito deaths.

Methods

This study was a true experimental (post test only control group design) design, with red ginger extract and fractionation (*Z. officinale* Roscoe *var. rubrum*) and 6 treatment concentrations of 0.5%, 1%, 1.5%, 2 %, 2.5% and 0% as control with aquadest. This research was conducted in June 2017 at MIPA Biology Laboratory Unari (extraction and fractionation of red ginger) and test of red ginger fractionation activity (*Z. officinale* Roscoe *var. rubrum*) to *Ae. aegypti* mosquito death done at Entomology Laboratory of Local P2B2 Baturaja Regency Ogan Komering Ulu (OKU).

Material used 7 kg red ginger (*Z. officinale* Roscoe *var. rubrum*), *Ae. aegypti* strain Liverpool, n-hexane, ethyl acetate and water methanol, aquadest, tissue, cotton, filter paper, plastic bags, 70% alcohol and aluminum foil. Tools used socket extraction, rotary vacuum evaporator, oven, microscope, blender, measuring cup, knife, scales, tweezers, portals, pipettes, test cage (30 cm x 30 cm), digital camera, scissors, sprayer and stationery.

The sample of *Ae. aegypti* mosquitoes used in this study amounted to 500 individuals, originating from the breeding process at the Entomology Laboratory of P2B2 Baturaja Local Laboratory of Ogan Komering Ulu Regency (OKU) with Liverpool strain. The sample of *Ae. aegypti* mosquitoes used in this research was female mosquito because it has long life and endurance.

Red ginger extract is made by washing 7 kg of red ginger and slice ginger thinly and dry for 5-7 days. After dry puree into powder and soak in methanol stir for ± 30 minutes and let stand for 2x24 hours. Strain the red ginger methanol extract and then insert it into an evaporated tube with a temperature of 80^0C until thickened or paste-shaped.

The fractionation was carried out by extract obtained into the funnel separator and 250 ml of n-hexane solvent (4 x 250 ml) plus aquadest: methanol water (1 : 1) 200 ml. The mixture was shaken for 5 minutes and allowed until separation occurs, then the dissolved fraction in n-hexane was separated from the residue. The water methanol fraction was followed by the addition of 1 liter of ethyl acetate in phases. Each time the 250 ml of ethyl acetate (4 × 250 ml) was added and then shaken for 5 minutes and allowed to break apart, the ethyl acetate fraction was separated from the residue. From the fractionation process obtained 3 fractions of the fraction of n-hexane, ethyl acetate and methanol water. The n-hexane fraction has a weight of 22.15 g heavier than water methanol which was 22.15 g and 20.73 g ethyl acetate. The extract and the three fractions were tested for efficacy by comparing extract, n-hexane fraction, ethyl acetate fraction and red ginger methanol (*Z. officinale* Roscoe *var. rubrum*) fractions against *Ae. aegypti* mosquitoes.

At the treatment stage, each test enclosure was inserted by *Ae. aegypti* mosquitoes as many as 20. Furthermore, in 5 test cages treated with different concentrations of 0.5%, 1%, 1.5%, 2%, 2.5% and 1 test cage as a negative control. This concentration test was performed with 4 repetitions. At the time of study the room temperature used was 29^0C and 70% humidity.

ISSN 2598 0580

After treatment, observation of mortality or death of *Ae. aegypti* mosquitoes from each concentration after 60 minutes of treatment. The results of mortality data were compared and analyzed by Analysis of Variance (ANOVA) which showed the difference of red ginger extract concentration had significant effect on *Ae. aegypti* mosquito mortality.

Results and Dicussions

Based on the results of the efficacy test of extracts, n-hexane fraction, ethyl acetate fraction and water methanol fraction of red ginger (*Z. officinale* Roscoe *var. rubrum*) fraction against *Ae. aegypti* mosquito in the following data:

Table 1. Efficacy Test of Ginger Extracts

Test Material	Amount Sample	% Amount	Dead Mosquitoes
Red ginger extract	20	2.5	11
N-hexane fraction	20	2.5	13
Ethyl acetate fraction	20	2.5	10
Water methanol fraction	20	2.5	4

The n-hexane fraction was a fraction with high insecticidal force killing *Ae. aegypti* mosquitoes of 13 (55%) at a concentration of 2.5% compared to the active fraction of ethyl acetate, water methanol and red ginger extract (*Z. officinale* Roscoe *var. rubrum*).

Based on the distribution of *Ae. aegypti* mosquito deaths treated with various concentrations of active n-hexane fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) for 60 minutes of observation, the following data were obtained:

Table 2. Mean Deaths of *Ae. aegypti* Mosquitoes

			Average	
(%)	Amount Mosquitoes	Total Dead	(n) Dead	(%) Dead

0.0	20	0	0	0
0.5	20	26	7	32.5
1.0	20	27	7	33.8
1.5	20	41	10	51.3
2.0	20	47	12	58.8
2.5	20	52	13	65.0

Table 2. shows that the treatment of various concentrations of active n-hexane fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) used in this study were 0.5%, 1.0%, 1.5%, 2%, 2.5% and 0% as controls, recpectively.

The result of observation for 60 minutes showed that various concentration of n-hexane active fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) had an effect on *Ae. aegypti* mosquito death. The most influential concentration of *Ae. aegypti* mosquitoe death was the concentration of 2.5% with the percentage of mortality rate 65%, while the lowest percentage of *Ae. aegypti* mosquito death was 0.5% with a percentage of 32.5%. The data proves that the higher the concentration given the higher the mortality value.

The average death rate of *Ae. aegypti* mosquitoes after treatment of various concentrations of active n-hexane fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) after 60 minutes, *Ae. aegypti* mosquito death was highest at 2.5% concentration and lowest at 0.5%.

Then the data was analyzed by One Way Anova test and showed the active n-hexane fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) had significant effect on *Ae. aegypti* larvae death with p value = 0.000 ($p < 0.05$).

Discussion

The number of dead mosquitoes varies at each treatment concentration. In general, the high concentration given would affect the number of dead mosquitoes. The number of dead mosquitoes increased significantly at concentrations of 0.5%, 1%, 1.5%, 2% and 2.5% causing the concentration of dead mosquitoes 32.5%, 33.8%, 51.3%, 58.8% to 2.5% concentration of dead mosquitoes reached 65.0%. Thus, the higher the concentration of active n-hexane fraction from red ginger extract (*Z. officinale* Roscoe *var. rubrum*) given, the greater the percentage of *Ae. aegypti* mosquito deaths. This was due to the higher concentration used, the higher the active compounds present in the active n-hexane fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*). Treatment of various concentrations of vegetable insecticides has toxic effects on insects.

There are several ways of entering toxic agent to the body of insects, among others through direct contact (contact poison) and breathing (fumigants).[12]How insecticides can enter the insect's body when the insects are in direct contact with insecticides, or insects running on the surface of plants that already contain insecticides. Insecticides enter into the body of the insect through the body wall and will be able to cause death on insects.[13]

The active compound contained in n-hexane from red ginger extract (*Z. officinale* Roscoe *var. rubrum*) is terpenoid. Terpenoid bioactive compounds can affect the nervous system or muscles, hormonal balance, reproduction, behaviors such as repellents, pullers, anti-feeding and respiratory system. Terpenoids include classes of volatile compounds such as flavanoids and essential oils.[14]

Flavanoid is a compound that can inhibit growth and work as a strong respiratory inhibitor and has a visible effect on the body cavity. The process of flavonoid work on *Ae. aegypti* mosquitoes when there is contact with the mosquito skin surface in the form of hemolymph resulting in damage to hemolymph cells.[15] Flavonoids interfere with cellular respiration by inhibiting the electron transport system in the mitochondria thus blocking the production of ATP and causing a decrease in oxygen function which can cause any neurological disorders and spiracle damage that ends in death.[16]

The benefit of flavonoids to insects is as an attraction of insects to pollinate and can be used as an active ingredient in the manufacture of vegetable insecticides. In insects, flavonoids act as respiratory inhibitors. Work by inhibiting the respiratory chain, inhibiting oxidic phosphorylation, or by disconnecting the chain between the respiratory chains and oxidative phosphorylation. Flavonoids are thought to interfere with energy metabolism in mitochondria by inhibiting electron transport systems. The presence of obstacles to the electron transport system would inhibit the production of ATP and cause a decrease in mitochondrial oxygen consumption.[16]

Essential oils are volatile oils that are not water-soluble from plants. The essential compounds (zingiberene, zingiberol and kaemferol) serve as receptors that damage the digestive tract, respiratory system, decrease the activity of insect feeding and as insecticides.

The smell of the essential oil fragrance will be detected by chemical receptors (chemoreceptor) found on the mosquito antenna and passed on to nerve impulses. The smell of this essential oil is not favored by mosquitoes. That's what is then translated into mosquito brain so that mosquitoes will express to avoid the source of odor.[17]

Essential oils work as contact poison through the surface of the mosquito body,[18] because phenol (eugenol) is easily absorbed through the skin.[19] Contact poison will enter the body of the mosquito through the cuticle so that if the insecticide direct contact on the skin then little by little insecticide molecules will go into the mosquito body. As time goes by the accumulation of insecticides into mosquitoes can cause death.[20]

Ginger generally contains volatile oil and non volatile oil compounds. The main content of ginger volatile oil is sesquiterpenes and monoterpenes. The sesquiterpene compounds contained are zingiberene (20-30%), ar-curcumene (6-19%), β-sesquiphelandrene (7-12%). While monoterpenes contained compounds such as α-pinene, borny acetate, borneol, camphene, p-cymene, cincol, citral, cumene, farnese, geranio, limonene, linalol, myreene, β-pinene and saninene. The non-volatile component consists of oleoresin consisting of shogaol and gingerol which gives a bitter, spicy flavor to ginger and is a phenolic antioxidant of ginger.[8]

Conclusion

There were significant differences and effects of various concentrations of active n-hexane fraction of red ginger extract (*Z. officinale* Roscoe *var. rubrum*) against *Ae. aegypti* mosquito deaths because *p-value* < α (0.00 < 0.05).

Acknowledgments

We would say thank you for Entomology Laboratory of P2B2 Baturaja Local Laboratory of Ogan Komering Ulu Regency (OKU).

References

1. Najmah. 2016. Epidemiologi Penyakit Menular. Penerbit CV Trans Info Media, Jakarta, Indonesia.
2. Kemenkes RI. 2011. Buletin Jendela Epidemiologi. Pusat Data dan Surveilans Epidemiologi KementerianK esehatan RI. ISSN-2087-1546.
3. Dinas Kesehatan Propinsi Sumatera Selatan. 2016. Profil Dinas Kesehatan Propinsi Sumatera Selatan Tahun 2015. Palembang, Indonesia.
4. Dinas Kesehatan Kota Palembang. 2015. Profil Dinas Kesehatan Kota Palembang Tahun 2014. Palembang, Indonesia.
5. Departemen Kesehatan RI. 2004. Pedoman Ekologi dan Aspek Perilaku Vektor. Direktorat Jenderal PPM dan PL Departemen Kesehatan RI, Jakarta, Indonesia.
6. Hamid, A, dan Y. Nuryani. 1992. Kumpulan Abstrak Seminar dan Lokakarya Nasional Etnobotani, Bogor. P.1. Dalam S. Riyadi, A. Kuncoro, dan A.D.P. Utami. Tumbuhan Beracun. Malang: Ballittas.
7. Heyne, K. 1987. Tumbuhan Berguna Indonesia, Jilid II dan III. Diterjemahkan oleh Badan Litbang Kehutanan Jakarta. Penerbit Yayasan Sarana Wana Jaya, Jakarta.
8. Suadnyani dan Sudarmaja. 2016. Pengaruh Konsentrasi Ekstrak Etanol Rimpang Jahe Merah (*Zingiber officinale* Roscoe) terhadap Kematian Larva Nyamuk *Aedes aegypti*. Fakultas Kedokteran Universitas Udayana. Vol. 5, No.8, Agustus 2016. ISSN: 2303-1395.
9. Kusumaningati, R.W. 2009. Analisis Kandungan Fenol Total Jahe (*Zingiber officinale*). Universitas Indonesia Jakarta, Indonesia.

10. Setyawan, B. 2015. Peluang Usaha Budidaya Jahe. Penerbit Pustaka Baru Press Yogakarta, Indonesia.
11. Rahajoe dan Mirzaqotul. 2012. Uji Potensi Dekok Rimpang Jahe (*Zingiber officinale*) sebagai Insektisida terhadap Lalat Rumah (*Musca domestica*) dengan Metode Semprot. Universitas Brawijaya Malang, Indonesia.
12. Hasibuan, R. 2015. Insektisida Organik Sintetik dan Biorasional. Penerbit Plantaxia Yogyakarta, Indonesia.
13. Setiawan, E., Siti Rabbani Karimuna, Jafriati. 2016. Efektivitas Ekstrak Biji Sirsak (*Annona muricata* L) sebagai Insektisida Alami terhadap Nyamuk *Aedes aegypt i*sebagai Vektor DBD. Fakultas Kesehatan Masyarakat Universitas Halu Oleo, Kendari, Indonesia.
14. Setyawaty, D. 2002. Studi Pengaruh Ekstrak Daun Sirih (*Piper battle* Linn) dalam Palarut Akuades, Etanol dan Metanol terhadap Perkembangan Larva Nyamuk *Culex quinquefascatus.* Fakultas Kedokteran Hewan, Institut Pertanian Bogor, Indonesia.
15. Robinson, T. 1995. Kandungan Organik Tumbuhan Tinggi. ITB Press, Bandung, Indonesia. Hal: 71-72, 157, 191-192, 208.
16. Aryanti, 2016. Pengaruh Fraksi dan Senyawa Aktif Daun Pare (*Momordica balsamina* L) terhadap Bakteri *Salmonella typhi* Penyebab Salmonellosis. Fakultas Kedokteran Universitas Sriwijaya Palembang, Indonesia.
17. Shinta. 2010. Potensi Minyak Atsiri Daun Nilam (*Pogestemoncablin* B), Daun Babadotan (*Ageratum conyzoides* L), BungaKenanga (*Cananga odorata* Hook & Thoms) dan Daun Rosemarry (*Rosmarinus officinalis* L) sebagai Repelan terhadap Nyamuk *Aedes aegypti*. Artikel pada Simposium Nasional Litbangkes Ke-6.
18. Ridhwan, M. dan Isharyanto. 2016. Potensi Kemangi sebagai Pestisida Nabati. Fakultas FKIP Biologi Universitas Serambi Mekkah, Aceh, Indonesia.
19. Wilbraham, M. dan M. Matta. 1992. Pengantar Kimia Organik dan Hayati. In S. A. Introduction to Organic and Biological Chemistry. Penerbit ITB Press, Bandung, Indonesia.
20. Wudianto, R. 1998. Petunjuk Penggunaan Pestisida. Penerbit: Penebar Swadaya, Jakarta, Indonesia.