



THE PHYTOCHEMICAL ANALYSIS OF HAY INFUSIONS AND PAPAYA LEAF JUICE AS AN ATTRACTANT CONTAINING INSECTICIDE FOR *Aedes Aegypti*

Widya Hary Cahyati¹✉, Widya Asmara², S. Rahmah Umniyati³, Budi Mulyaningsih³

¹Public Health Department, Sport Science Faculty, Universitas Negeri Semarang

²Faculty of Veterinary, Universitas Gadjah Mada

³Faculty of Medicine, Universitas Gadjah Mada

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Abstract

Aedes aegypti mosquito population could be controlled by using lethal ovitrap. The addition of hay infusions as a attractant greatly enhance *Aedes aegypti* eggs trapped, and papaya leaf juice may inhibit *Aedes aegypti* eggs evolve to larvae or a larvae to pupae stage. This study was conducted to find out the chemical compounds in hay infusion and papaya leaf juice. We used phytochemical test using UV-Vis Spectrophotometry, Thin Layer Chromatography, and High Performance Liquid Chromatography (HPLC) method. The results showed that hay infusion contains 12,75 mg/L ammonium and <1,20 ppm ($\mu\text{g}/\text{mL}$) lactic acid and papaya leaf juice contains 0,25% alkaloid, 0,14% flavonoid, 0,30% saponin, ≤ 68 mg/L steroid and 11,34% tannin, but negative terpenoid. We concluded that hay infusion and papaya leaf juice contains chemical compounds that could be use as attractant and bioinsecticide to *Aedes aegypti*, respectively.

Introduction

Nowadays, a wide range of tropical diseases transmitted by mosquitoes. Malaria infection is transmitted by Anopheles mosquito and dengue is transmitted by *Aedes aegypti* mosquito. Main problem facing Indonesia currently is high number of people infected by Dengue Hemorrhagic Fever (DHF). In 2012, the number of dengue patients that are reported is 90.245 cases with 816 mortality (Incidence Rate= 37,11 per 100.000 population and CFR =0.90%). Dengue cases increased about 112.511 cases in 2013 and morbidity rate recorded is 45.85 per 100,000 population with a mortality rate about 0.77% (871 deaths), and

in 2014 the cases recorded about 100.347 cases with a mortality rate about 907 (DEPKES RI, 2015).

Due to increasing number of dengue cases each year among diverse regions, the government should take appropriate action to control the transmission of the disease. To break the dengue transmission chain, we could eradicate or control the disease through the transmission source (vector), which is *Aedes aegypti* mosquito. Vector control of dengue fever so far have been conducted with dengue vector surveillance system, fogging with insecticide on where cases found, and using larvicides (Astuti, 2011). There are several

✉ Correspondence Address:

Jurusan Ilmu Kesehatan Masyarakat, Gedung F5 Lantai 2
Fakultas Ilmu Keolahragaan, Universitas Negeri Semarang
Email : widyahary27@mail.unnes.ac.id

methods of surveillance to detect infestation of *Aedes aegypti* in certain areas, such as larvae-stage survey, adult mosquitoes survey, and egg-stage survey. Egg stage survey have been proved to be effective for detecting the presence of *Aedes aegypti* infestation in certain areas. Egg stage survey usually use ovitrap (oviposition trap /eggs traps). Use of ovitrap have been succeed in reducing vector density in some countries. This method has been successfully applied in Singapore by installing 2.000 ovitrap in the DHF endemic area. Ovitrap is designed to attract gravid female mosquitoes to lay their eggs and then counted and identified (Astuti, 2011).

Ovitrap used for surveillance of *Aedes aegypti* could be modified to kill its adult or pre-adult population. Modifications of ovitrap become mosquito trap could be made by adding some insecticide in the spawn media (*ovistrip*). *Lethal ovitrap* with insecticidal *cypermethrin* are able to reduce the density of mosquitoes due to mosquitoes which want to laying eggs there will in contact with *ovistrip* containing insecticide and its eventually die. However, insecticide with *cypermethrin* have moderate risk or are grade II toxin (Ramadhani, 2013). Therefore, it is required an insecticide that derived from a non-chemical compounds or a natural resources.

Natural insecticides can be made from several parts of plants, like roots, tubers, stems, leaves, seeds and fruits with simple technology, such as juice, infusion, extract, and stew (Elimam, 2009). Plant that can be used as insecticide is papaya (*Carica papaya L.*). Papaya leaf has chosen as an alternative natural insecticide due to its occasionally used maximally by the public. Most people only eat the fruit with papaya leaver left behind. The plant-based insecticide have some benefit compared to chemical insecticides, including rapid degradation or decomposition by sunlight, air, moisture and other natural components, thereby reducing the risk of soil and water contamination. In addition, the plant-based insecticides generally has a low toxicity to mammals, thereby plant-based insecticide may applied safely for humans being. Selection of materials to be used as an insecticide must be safe for humans and other organisms. Beside,

this material is also easily obtained, and is expected to have a positive impact on human health (Pratiwi, 2012).

Papaya leaves contain active substances such as papain, carpaine alkaloids, flavonoids, tannins, karposid, and saponins (Milind, 2011). Other study showed that papaya leaves contain alkaloids, triterpenoids, steroids, flavonoids, saponins and tannins (A'yun, 2015). Akhila and Vijayalakshmi found that juice of papaya leaves contain alkaloids, phenols, flavonoids and amino acids (Akhila, 2015). The active substances contained in papaya leaves can affect some physical activity of insects, such as inhibition of feeding, breathing, growth and development, until cause death.

Modifications ovitrap by adding attractant substances are proven increasing the amounts of eggs trapped. Mosquito attractant is an alluring substance that attract mosquito come to attractant be placed. Attractants can be derived from plants that are easily found around or other scent substance that can attract mosquitoes to lay eggs. Among the several types of plants that have been tested and shown to be significant as the attractant is hay infusion. *Lethal ovitrap* with the addition of hay infusion produce higher larval mortality compared to attractand made by used-colonization water (Salim, 2015).

Based on background above, we aim to find out active compounds of hay infusion which serves as attractants and active compounds in papaya leaf juice that serves as an insecticide.

Method

Phytochemical analysis of hay infusion and papaya leaf juice were held in March 2016 at Integrated Research and Testing Laboratory Universitas Gadjah Mada. Papaya leaf and hay samples were obtained from Semarang city. The papaya leaf disintegrated with blender. These samples then analyzed with chromatography and UV-vis spectrophotometer.

Making hay infusion required 125 grams of dried hay and then cut into small pieces and soaked in 15 liters of water for 7 days, then filtered. The infusion result were then mixed with freshwater (e.g. well water) until the desired concentration (Polson, 2002). This study used hay infusion with 10% concentration.

Making juice of papaya leaves took 1,000

grams of papaya leaves. Papaya leaves were picked in the morning around Semarang city. Papaya leaves then blended with 1 liter of water and soaked for 1 hour, then filtered.

Phytochemical test performed on a hay infusion and papaya leaf juice. Analysis of ammonium and lactic acid of hay infusion was using UV-vis spectrophotometry and HPLC method, respectively. Papaya leaf juice was analyzed using UV-vis spectrophotometric method for analysis of total alkaloids quinine equivalent, total equivalent of routine flavonoid, saponin from Quillaja bark quantitative, and total tannin equivalent tannic acid. Analysis of terpenoids and steroid equivalent beta sitosterol were using thin layer chromatography (TLC).

UV-vis spectrophotometry is a method of analysis that is wide utilization, both for qualitative and quantitative analysis (Ardianingsih, 2009). Thin Layer Chromatography is performed by separation of mixtures of compounds with stationary phase and a mobile phase with thin layer of adsorbent material. This method is quick and easy to perform and only requires absorbent and very small portion with better separation result (Ardianingsih, 2009). *High Performance Liquid Chromatography* (HPLC) is performed by using high pressure to send mobile phase into the column (Ardianingsih, 2009).

Results and Discussion

The phytochemical test results showed that hay infusion contain 12.75 mg/L ammonium (NH₄) and <1.20 ppm (µg / mL) lactic acid. Ammonium and lactic acid in the hay infusion are known have an effect on olfactory nerves of *Aedes aegypti*. Hay infusion-baited ovitrap shown to increase the number of eggs caught eight times more than the standard ovitrap (Polson, 2002). Study conducted by Salim and Satoto showed that the ovitrap contain hay infusion yield the highest number of eggs trapped compared to other media like

used-colonization water or distilled water. This proves that the attractant with hay infusion affect the oviposition process. Other study also showed that *Aedes sp* mosquito trapped more on ovitrap with hay infusion-baited attractant which placed outside and inside the house compared to ovitrap with plain tap water (Dwinata, 2015).

In general, there are three kind of attractants that attract mosquitoes, host odors, pheromones, habitat attractants. Host odors are released by human body or other animals. Pheromones come from eggs that had been laid and evaporates from outer thin layer of eggs for 48 hours after oviposition. Pheromones will attract another gravid female mosquitoes to lay their eggs in these place, usually these place contain abundant organic material. Habitat attractants are chemical compounds that released by soaking a plant parts or animal parts for example hay infusion (Dwinata, 2015).

Hay infusion undergo metabolic processes that produce ammonia and CO₂. Hay infusion containing about 3.74 mg/L ammonia, 23.5 mg/ L CO₂, 18.2 mg/L lactic acid, 1.6 mg/L octenol, and 17.1 mg/L fatty acids. These substances are able to stimulate *Aedes sp.* olfactory nerve and may respond by laying eggs in that place (Elimam, 2009). The large amounts of eggs found in hay infusion may be influenced by many gravid female mosquito attracted and stimulated to lay their eggs in that place, therefore hay infusion can be considered as oviposition attractants. A substance is considered as attractants and oviposition stimulants if it causes gravid female mosquitoes approaching the source of the substance and stimulated to lay her eggs there (Cania, 2013).

The oviposition process of adult mosquito consist of two phases, pre-oviposition and oviposition phase. In pre-oviposition phase, adult mosquitoes seeking, locating and selecting for appropriate oviposition place. While, oviposition phase is the process of laying the

Table 1. Phytochemical Analysis of Hay Infusion

No.	Test parameter	Result	Unit	Method
1.	Amonium (NH ₄)	12,75	mg/L	UV-vis Spectrofotometry
2.	Lactid acid	<1,20	ppm (µg/mL)	HPLC

Source : Research Data, March 2016

Table 2. Phytochemical Analysis of Papaya leaves juices

No.	Test Parameter	Result	Unit	Method
1.	Total Alkaloid Equivalent Quinine	0,25	% b/v	Spectrophotometry UV-vis
2.	Total Flavonoid Equivalent Rutin	0,14	% b/v	Spectrophotometry UV-vis
3.	Terpenoid	-	-	KLT
4.	Saponin Quillaja Bark Quantitative	0,30	% b/v	Spectrophotometry UV-vis
5.	Steroid Equivalent Beta Sitosterol	≤68	mg/mL	KLT
6.	Total Tannin Equivalent Tannic Acid	11,34	% b/v	Spectrophotometry UV-vis

Resource : Research Data, March 2016

eggs in that place (Cania, 2013; Harfriani, 2012; Alfiah, 2012). In pre-oviposition phase, *Aedes sp* using diverse sensory organs to evaluate signs of surrounding (physical and chemical) to make sure appropriate place for oviposition (Cania, 2013; Sofiana, 2016; Wanti, 2016).

In seeking phase, the mosquitoes use long-distance visual signs like sunlight, moisture, color, temperature and odor of target place. When mosquito getting close to the target place, they use short-distance visual signs such as odor and taste like volatile substances contained in the oviposition place or the chemical compounds in the water. Hay infusion release chemicals such as ammonia and CO₂. The visual signs are received by receptors of mosquito, so mosquitoes decide to lay eggs on ovitrap with hay infusion (Cania, 2013).

Increasing oviposition of *Aedes aegypti* cause by non-volatile substances on the surface of hay infusion. When mosquito chemotactile sensory organs interact with it, it will stimulate the mosquito to lay her eggs, compared to odor that attracts mosquitoes from afar (Ponnusamy et al., 2010). These chemical substances may produced from fermentation process of microorganisms in hay infusion. Attractivity of organic infusion is influenced by the bacteria growth in the infusion which also increases the production of secondary metabolites (Santos, 2010).

Phytochemical test of papaya leaf juice

using UV-vis spectrophotometry resulted that papaya leaf contains 0.25% alkaloids, 0.14% flavonoids, 0.30% total saponins and 11.34% tannins. Phytochemical test of papaya leaf juice using Thin Layer Chromatography (TLC) resulted that papaya leaf also contains ≤68 mg/mL steroid, but negative terpenoids. This is consistent with the research committed by (A'yun, 2015) where papaya leaves contains alkaloid, triterpenoid, steroid, flavonoid, saponin and tannin. Other studies also have shown that papaya leaves juice contains alkaloid, phenol, flavonoid and amino acids (Akhila, 2015).

The chemical substances present in the juice of papaya leaves, such as flavonoid, tannin, saponin, steroid and alkaloid function as natural insecticides and are entomotoxicity. These chemicals can inhibit the eggs so it wouldn't hatch or inhibit organ development process from egg to larval instar 1, result in abnormal development, and larvae won't survive, and eventually die (Chaieb, 2010). Papaya leaf juice also contains juvenile-like hormone that influence endogenous juvenile hormone level of *Aedes aegypti*, causing abnormal development and also interrupt hatching eggs process (Astuti, 2011).

Inhibition of egg hatchability of *Aedes aegypti* are presumed occur due to entry of insecticide into the outer layer of egg through diffusion, pass through polygonal points that

covering entire surface of the egg. Diffusion occur due to water potential difference between insecticide outside the egg (hypertonic) and water inside the egg (hipotonic). This insecticide inside the egg will disturb metabolic processes and lead to a wide range damages to the egg (Aulia, 2014).

Flavonoids and alkaloids resemble juvenile hormone that have effect on insect development from egg to larva (Elimam et al., 2009). According to research flavonoid cause *Aedes aegypti* egg failed to hatch into larvae. Alkaloid inside papaya leaf juice can stimulate certain endocrine glands to produce ecdyson hormones. Increasing these hormones could cause metamorphosis failure (Akhila, 2015).

Research conducted by (Salim, 2015) showed that the larvae newly hatched (larval instar 1) has a vulnerable body, their organs are not ready to function effectively, therefore may not survive when exposed to natural insecticide containing alkaloids. The head of newly hatched larvae is still creased, triangles and no wider than its thorax, the comb at the labrum for feeding still tucked in preoral cavity. The larvae will soon suck water to contract the thorax and abdomen, pushing haemolymph toward the head and it will enlarge wider than the thorax, expansion mainly occur in the frontal part. Furthermore, the head will grow wider and darker. Feeding comb will grow and ready to function. These conditions proved that alkaloid inhibit normal organ development in larval instar 1, so that development become incomplete, the larvae may not survive and eventually die.

Saponin are *entomototoxicity* that could inhibit development of eggs into larvae by disturbing egg membrane so other substances can enter the egg and causing developmental abnormality, and lead to hatch failure (Aulia, 2014). Saponin will bind to the aglycone of flavonoids that act as *ecdyson blocker* that also act as a inhibitor to egg development into larvae (Astuti, 2011).

Steroid in papaya leaf juice has a protective function, for example as fitoecdison which has a structure similar to an insect molting hormone, so steroid may inhibit the process of molting process when ingested (Ardianingsih, 2009). The presence of steroids

will affect thickening of chitin layer, causing body abnormality. Steroid cause increase rate of cell elongation in larval mortality with steroid treatment (Mardiana, 2009).

Chemical compounds in the papaya leaf juice may also serve as larvicides. Research conducted by (Valiant et al., 2010) showed that papaya leaf infusion contain alkaloid, tannin, flavonol, and papain have effect as larvicides against the mosquito *Culex sp.* Alkaloid and steroid proved causing *Aedes albopictus* larvae die (Astuti, 2011). Papaya leaves extract contain alkaloid carpaine, papain enzyme, tocophenol, flavonoid, saponin and tannin also have effect as larvicides causing Anopheles larvae die (Aulia, 2014). Other research also showed that alkaloid, flavonoid, saponin, tannin, sterol and triterpene have effect as larvicides to *Aedes aegypti* larvae (Widawati, 2013). These studies confirmed that the chemical compounds such as alkaloid, tannin, saponin, steroid and flavonoid contained in the juice of papaya leaves serves as larvicides.

Alkaloids often toxic to human but many of them have major physiological effects, hence its widely used for pharmacological purpose. Alkaloids have very diverse structure and show broad range of pharmacological effects including antimicrobial effect. Alkaloid are a salt, so it can interfering cell membrane of digestive tract and damage the cell and may also disturb larval nervous systems by inhibiting enzyme acetylcholinesterase action. Alkaloids make this enzyme failed to transmit stimulus to larval digestive tract (midgut), causing gut movement could not be controlled. Damage to the central nervous system may inhibit the cholinesterase enzyme activity and acetylcholine will accumulate, so it will block all the impulses of central nervous system to the muscles and eventually causing death (Salim, 2015).

Tannin are one of polyphenols that contained in papaya plants. If tannin contact with the tongue, precipitation reaction will occur and tasted as astringent taste. Tannin are found in various woody plants and herbs, served as plant defense mechanisms by blocking the insect to digest the plant (Chaieb, 2010).

Tannin are polyphenolic compounds that form a complex compounds if interact with

protein. Tannin may interfere insect digestion by binding to proteins in the digestive tracts that required by insects for growth, causing abnormal digestion (Yunita., 2009) . Tannin works by activating cell lysis due to activation of proteolytic enzymes inside the cell by tannin. The complex produced by the interaction of tannin with protein are toxic, that may play a role in inhibiting growth and reduce insects appetite through inhibiting digestive enzymes activity. Larval growth rate reduction and nutritional deficiency will occur as response to tannin (Cania, 2013).

Saponin are glycosides compound inside plant which resembling soap and are water soluble. Saponin are terpenoid compound which able to bind free-sterol in the digestive tract. Because its effect reducing free-sterol, it will affect the process of moulting of insects (Chaieb, 2010). Saponin are found in all parts of the papaya plants such as roots, leaves, stems, and flowers. Saponin will form a foam when shaken with water that may lowering the surface tension so it will damage insect cell membrane, and saponin have also bitter taste (Salim, 2015).

Saponin are known as insecticide, because the saponin contained in food consumed by insect can lowering digestion enzyme activity and absorption of food. Effect of saponin seen in outer part of insects (cuticle), which it wash out wax layer that protect the insect body and will causing death from loss a lot of body fluids. Saponin may also enter through the respiratory organs and cause the cell membrane damage or metabolism impairment (Santos, 2010).

Flavonoid are one of the toxic compounds contained in papaya leaves. Some typical properties of flavonoids are have a very sharp odor, bitter taste, soluble in water, an organic solvents, and also easily denaturate at high temperature (Cania, 2013). Flavonoid have several benefit. First, for plants, is a plant regulator, photosynthesis regulator, antimicroba and antiviral agent. Second, for human being, such as an antibiotic against cancer and kidney disease, and inhibit bleeding. Third, for insects, such as an attraction agent for pollinating insects. Fourth, other uses as an active compounds in the manufacture of plant-based insecticide (Chaieb, 2010).

Flavonoid is plant defense compounds

that may inhibiting insect feeding and are also toxic substance. Flavonoid work as a potent inhibitor of respiratory due to its toxic to respiratory system. Flavonoid works by entering larval body through the respiratory system, which then will cause paralysis of nerves, respiratory system damage and breathe impairment and death. Change of larval body position from normal position could be caused by flavonoid ingested through siphon causing damage, so larvae would align with the surface of the water to make it easier to take up oxygen (Cania, 2013)

Conclusion

Phytochemical analysis of hay infusion and the papaya leaf juice showed that hay infusion contain 12.75 mg /L ammonium (NH₄) and <1.20 ppm (mg / mL) lactic acid. These substances could be use as an attractant for mosquitoes *Aedes aegypti*. Juice of papaya leaf contain 0.25% alkaloid, 0.14% flavonoid, 0.30% total saponin, 11.34% tannin, and ≤68 mg/mL steroid, but negative terpenoid. These substances could be serve as a natural insecticide as well as larvicide, and is *entomototoxicity*.

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