



# Potential Ectoparasiticide for Dog and Cat Fleas; a Combination of *Ficus Minahassae* Extract and Latex from *Carica Papaya* L.

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## ABSTRACT

The purpose of this study was to examine the chemical composition of mixed extracts of Langusei fruit (*Ficus minahassae* L.) and papaya latex (*Carica papaya* L.), as well as to conduct bioassays on fleas of the Ctenocephalides genus. LC-MS/MS was used to determine the combined extract's chemical makeup. The toxicity of the extract combination was tested in vivo on cat and dog flea imago. The phytochemical screening of Langusei extract and papaya latex yielded all of the major phytochemicals. The LCMS/MS results revealed that the combination of Langusei extract and papaya latex contains six chemicals. Four substances were identified: 3-butenyl glucosinolate, erythromycin, aluminum palmitate, and henpentakontilbenzena. Four compounds indicate a novel chemical. The combined extract was most lethal to both Ctenocephalides felis and *C. canis* in the P4 treatment (10%), with an average death of 100%, matching the control mortality of the synthetic insecticide deltamethrin. *C. felis* had the best LD50 in the F1 formula (4.003 mg/L), while *C. canis* had it in the F3 formula (3.733 mg/L). According to the findings, the combination of Langusei fruit extract and papaya latex may include novel chemicals. These chemicals are highly poisonous to Ctenocephalides ticks. As a result, it has the potential to be used as an ectoparasiticide for Ctenocephalides ticks.

**Keywords:** *Carica papaya*; *Ctenocephalides* sp; *Ficus minahassae*; Latex; Toxicity

## 1. Introduction

Cat and dog fleas (Ctenocephalides) are the most impactful ectoparasites found in dogs and cats worldwide. *Ctenocephalides* sp. is a mandatory haematophagous ectoparasite of

wildlife, domestic cats, and dogs worldwide. Ctenocephalides have a high reproductive rate, making them a significant health problem in dogs and cats [1, 2]. As animals are mostly cared for by humans, dogs and cats have close

contact with humans daily. *Ctenocephalides felis* and *Ctenocephalides canis* are the most common fleas infecting not just dogs and cats but also other warm-blooded animals, including humans [3]. *Ctenocephalides felis* is the most common species worldwide, with significant infestation rates. Because of their excellent resilience to a wide range of climatic circumstances, they have a cosmopolitan distribution [4]. *Ctenocephalides* spp can cause allergic dermatitis (FAD) and transmit various pathogenic bacteria to humans. Metagenomic analyses carried out in previous studies showed that many bacterial species associated with *Ctenocephalides* spp. have never been reported [5, 6].

Chemical insecticides are widely used to eradicate *Ctenocephalides* spp in dogs and cats. Resistance to cyfluthrin and permethrin insecticides have been reported in *Ctenocephalides* spp. Resistance also occurs to pyrethroid class insecticides [7, 8]. Fipronil-based pesticides should be examined for use on dog and cat fleas, according to research conducted in the United Kingdom. [9]. A combination of plant extracts is widely used to control paint fleas. The combination of cassia, thyme and oregano essential oils has an insecticidal effect on adult cat flea stages [10]. On cat and dog fleas, essential oils from *Illicium verum* and *Pelargonium graveolens* had insecticidal efficacy of more than 70%. [11]. In in vitro studies, the essential oil of *Ocimum gratissimum* was effective against all stages of ticks, producing adulticidal ( $LC_{50} = 5.85 \text{ g cm}^2$ ), ovicidal ( $LC_{50} = 1.79 \text{ g cm}^2$ ), and larvicidal ( $LC_{50} = 1.21 \text{ g cm}^2$ ) mortality at low concentrations [12]. *C. sativa* essential oil exhibited insecticidal activity (100% mortality at the highest concentration) for flea control at egg, larval, pupal and adult stages, with an  $LC_{50}$  of  $32.45 \mu\text{g/cm}^2$  [13].

The insecticidal potency of the combination of papaya latex extract and *Ficus minahassae* fruit has never been reported. Papaya latex contains many secondary metabolites, such as alkaloids, steroids, saponins and proteases, which have

insecticidal activity [14, 15]. Papaya latex has vigorous insecticidal activity on *Rhipicephalus microplus* and *Aedes aegypti* [16]. In contrast, Langusei (*Ficus minahassae*) has been used empirically by the Minahasa community as a raw material for botanical insecticides. However, the analysis of the bioactive content of the combination of papaya latex extract and *Ficus minahassae* has never been reported. Combining plant extracts and papaya latex can increase toxicity against insects [14, 17]. Furthermore, bioassays on insects, especially on dog and cat fleas, have not been reported. The chemical composition of the combination of *Carica papaya* latex extract and *Ficus minahassae* fruit was analyzed and determined to be the optimum ectoparasite concentration against ticks of the *Ctenocephalides* genus.

## 2. Materials and Methods

### 2.1 Plant sample collection

Langusei fruit was obtained from the forest of Mount Klabat, North Minahasa district (1027'12" N and 125001'41" E). In contrast, latex papaya was obtained from a local papaya plantation, Matungkas, Dimembe District (1.4842° N and 124.9749° E), North Minahasa Regency, North Sulawesi. Langusei fruit was determined in the biology laboratory of the Faculty of Mathematics, Natural and Earth Sciences, Manado State University. Papaya latex used was papaya fruit latex and preserved in sterile sample bottles, stored in a box at 25°C.

### 2.2 Cat and dog flea sample collection

*C. felis* and *C. canis* were obtained from North Minahasa Regency, Tomohon City (01° 18' 51" N and 124° 49' 40" E) and Minahasa Regency (1° 22' 44" N/ 124° 33' 52" E to 1° 01' 11" N/ 124° 54' 45" E to 125° 04' 21" E/ 1° 20' 25" L.U), Sulawesi Utara, Indonesia. Each location's sample consisted of 50 adult cats and dogs. Additionally, 50 dog and cat ticks were gathered from each location. Cat fleas and dog fleas were preserved alive and immediately brought to the laboratory for bioassays.

### 2.3 Extraction and phytochemical screening

Langusei fruit was extracted in the form of wet simplicia. Simplicia preparation was done using a fine blender. The simplicia was macerated using 90% alcohol (Kimia Farma). Ratio of simplicia and solvent was 1:4 (w/v) [15]. Then, 250 grams of simplicia were macerated with 1000 ml of 90% alcohol in a sterile glass container. Maceration was carried out at room temperature for 72 hours, stirring every hour to maximize extraction. One day before filtering, the macerate was placed in a Memmert incubator at 45 rpm. Then, the mixture was filtered using Whatman 41 filter paper, the filtrate was separated, and the dregs were followed by maceration again according to the previous procedure. The filtrate was then evaporated using a Heidolph rotary evaporator at 45°C, 50 rpm until a semisolid and concentrated crude extract were obtained. Screening for phytochemical groups was done using the Harborne method. The intensity of the content was assessed by comparing the color and precipitate generated to the control [17].

### 2.4 Analysis of compound content with LC-MS/MS

The compound content of the combination of Langusei fruit extract and papaya latex was analyzed using the Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [18, 19]. The combination of Langusei extract and papaya latex (1.4 mg) was dissolved in 100 mL methanol. The solution was filtered using a 0.2 µm GHP filter and injected into the UPLC system. The LC-MS system used was Xevo-ToF-1, which was equipped with a C-18 column (Particle dimensions 1.8 µm, 2.1×100 mm<sup>2</sup>), and MS with Xevo G2-S resolution, acquisition mode QTOF ESI (-) and MSE. The eluent consisted of 0.1% formic acid in distilled water (A) and 0.1% formic acid in acetonitrile (B). The total running time was 20 minutes at 100°C. The elution system was run with gradient elution at 0-1 min. The ratio of solvent A was 70%, and solvent B was 30%; at

6-18 minutes, solvent A was 5% solvent, B was 95%. At 19-20 minutes, the solvent was a linear gradient elution A 70% solvent B 30%. Furthermore, data processing was performed using MassLynx software. The findings of the LC/MS-MS data analysis are shown as chromatograms, allowing the number of compounds in each sample to be determined.

### 2.5 Ectoparasiticide bioassay

#### 2.5.1 LD<sub>50</sub> determination

The test solution was prepared using a combination of Langusei extract and papaya latex. Formula 1 was Langusei extract and papaya sap at a ratio of 1:1 (w/v). Formula 2 was Langusei extract and papaya sap at a ratio of 1:2 (w/v). Formula 3 was Langusei extract and papaya sap at a ratio of 1:3 (w/v). Each formula was tested on 10 *C. canis* specimens on cotton-lined Petri dishes. The test concentrations used were 5 mg/L, 15 mg/L, 45 mg/L, and 65 mg/L. Each test concentration was carried out in triplicate. The solution of each formula was sprayed on *C. canis* imago every 6 hours for 24 hours. *C. canis* imago were declared dead if there was no movement response after being given a stimulus or a gentle touch using a pin. The same method was applied to *C. felis*.

#### 2.5.2 Ectoparasiticide test

Preparation of the test solution utilizing a combination of previously prepared extracts was as follows: Three groups of test solutions were made. Preparing the test solution used Tween 80.2% (v/v) solvent to increase the solubility of the extract. Tween 80.2%, 2 ml added with 98 ml of distilled water. A 2.5% (w/v) concentration test solution was prepared by weighing 2.5 g of each combination of extracts (F1, F2, and F3) and then putting it into a volumetric flask and adding Tween 80 solvent (2%) until the volume reached 100 ml. The same steps were carried out to prepare the extract solution with a test concentration of 5% (w/v) and 10% (w/v). The test solution made was then put into each sprayer which had been labelled.

During the preparation stage, the petri dish container was given a cotton pad to cover it uniformly at the bottom, then was sprayed evenly with each test solution. The spraying was done three times.

Furthermore, *C. felis* and *C. canis* imago were divided into 4 treatment groups each with three replications. Each Petri dish contained ten fleas. Each group was sprayed, namely P0 as a control with 2% Tween 80 solvent, P1 as a positive control with deltamethrin 0.5EC (Butox 50®) concentration of 0.05%, and groups P2, P3, and P4 sequentially with ethanol extract of each plant concentrations of 2.5%, 5% and 10%. After being treated, observations were made on the number of dead fleas based on the predetermined observation time. In general, fleas will always move actively on the Petri dish, and fleas were declared dead if there was no response to movement after being given a stimulus or a gentle touch using a pin. Observations to see the number of fleas killed were carried out at 1 minute, shortly after treatment until the first 1 minute, then at 15, 30 and 60 minutes. The number of dead fleas was assessed to determine death rate due to the insecticidal activity of the extract combination. The same procedure was carried out on *C. felis*.

## 2.6 Research data analysis

Data from LC-MS/MS analysis are interpreted descriptively. The molecular weight of the LC-MS/MS output was used to search for the most similar compound in two online organic compound databases, namely the National Institute of Standards and Technology, USA (NIST:

<https://webbook.nist.gov>) and Advanced Industrial Science and Technology, Japan (AIST: <https://sdfs.db.aist.go.jp>). Data on variance in mortality of ticks from the genus Ctenocephalides were analyzed using the Duncan Multiple Range Test (DMRT) to determine if there were significant differences between treatments. Mortality data was used to determine Lethal Dose 50 (LD<sub>50</sub>) by probit analysis. Statistical analysis was performed using IBM SPSS Program 25.

## 3. Results and Discussion

### 3.1 Combination of extracts

The ethanol extract of Langusei fruit has a blackish-brown colour. The weight of the extract obtained was 33.8 g, with a yield of 12.6%. Papaya sap is milky white with a distinctive papaya aroma. The papaya latex was tapped from young papaya fruit, then preserved in sample bottles, and stored in the refrigerator at 25° C. The Langusei ethanol extract was then combined with papaya latex. Phytochemical screening showed that the combination of extracts contained all the main phytochemical groups. However, based on the intensity of the colour and precipitate, the ethanol extract of Langusei contained alkaloids, flavonoids, saponins and tannins at a higher intensity than steroids and triterpenoids. Phytochemical screening on the combination of Langusei extract and papaya latex showed increasing intensity differences in steroid and triterpenoid content. On the other hand, the content of saponins and tannins decreased compared to before being mixed with papaya latex (Table 1).

**Table 1.** Extraction results and phytochemical screening.

No.	Samples	Extract Weight (gr)	% yield	Phytochemical screening						Method
				A	F	S	T	St	Tr	
1	Langusei Extract	33,8	12,6	++	++	++	++	+	+	Harborne Method
2	Combination of Langusei Extract and Latex of Papaya	Ratio 1:1 (w/v)		++	++	+	+	++	++	Harborne Method

Description: A: Alkaloids, F: Flavonoids, S: Saponins, T: Tannins, St: Steroids, Tr: Triterpenoids  
 +: indicates the intensity of the content based on colour and precipitate

## 3.2 LC-MS/MS analysis of combination langusei extract and papaya latex

### 3.2.1 LC analysis

According to the LC results, the mixture of Langusei extract and papaya latex consists of six compounds, which can be seen

at the retention numbers 1.103, 1.258, 1.606, 4.299, 5, 450 and 7.784 m. The compound with the highest retention was 1.60, subsequent to 1.103, while the compound with the lowest retention was 4,299 (Fig. 1). Chemical substances isolated by LC were then evaluated using MS.

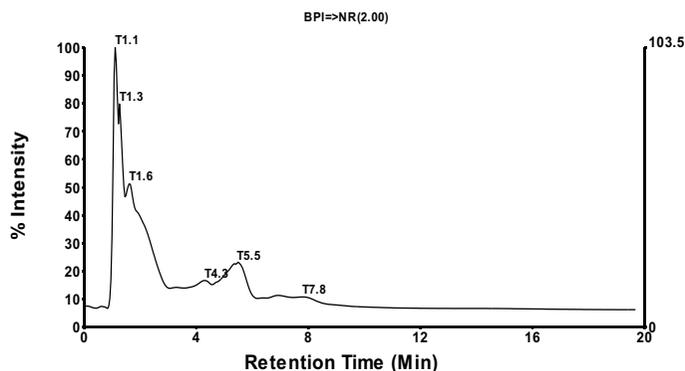


Fig. 1. LC Profile Mixture of Langusei extract and latex of papaya.

### 3.2.2 MS analysis

1. Retention Compound 1.103: As shown in the graph below, compounds having a retention of 1.103 exhibit fractionation. According to the findings, the molecule with a retention of 1.457 has a molecular weight of 733.76 (Table 2). A search for molecular weight data on the NIST Chemistry WebBook SRD 69 obtained a similar compound based on molecular weight, namely 3-butenyl

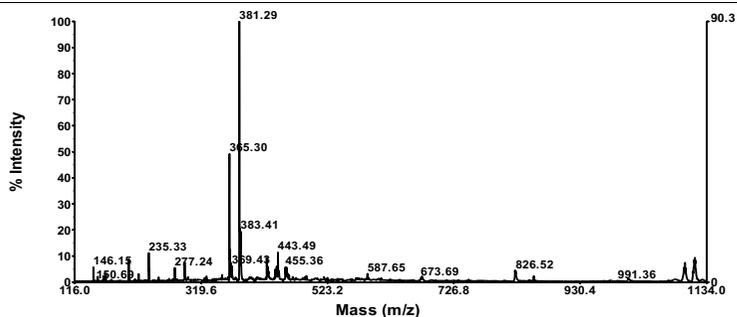
glucosinolate, TMS (C<sub>26</sub>H<sub>59</sub>NO<sub>9</sub>S<sub>2</sub>Si<sub>5</sub>). Index and relative intensity are shown in Appendix 1.

A search for compounds based on molecular weight at [https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct\\_frame\\_top.cgi](https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi) found that the closest compound is (-)-erythromycin (C<sub>37</sub>H<sub>67</sub>NO<sub>13</sub>) with molecular weight of 733.9 (Table 2).

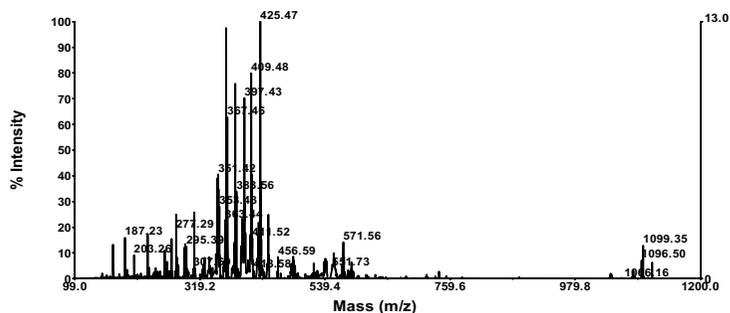
Table 2. Compound retention time and MS Profile.

No	Compound Retention time	MS Profile
1	1.103	

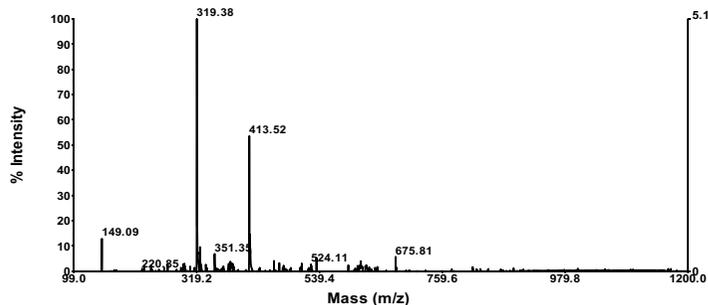
2 1.128



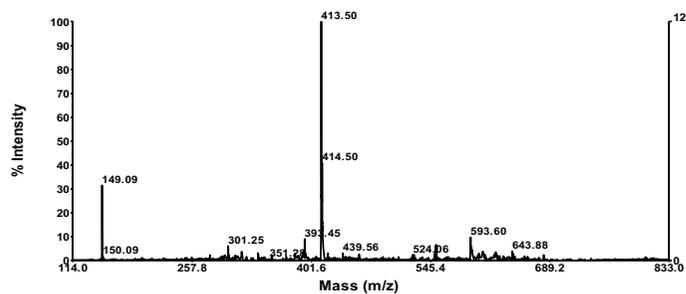
3 1.606



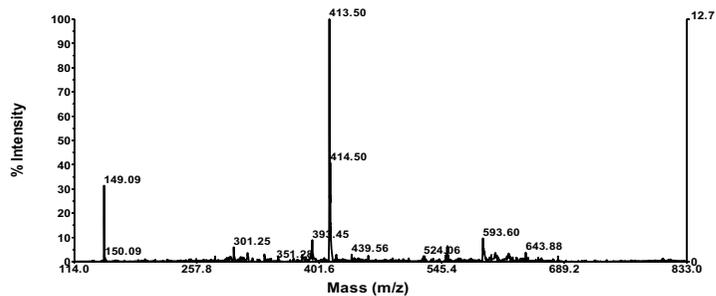
4 4.299



5 5.450



6 7.784



2. Compound Retention 1.1258: As is apparent in the graph below, compounds with a retention of 1.1258 generate fractionation. According to these findings, a molecule with a retention of 1.60 has a molecular weight of 733.76 (Table 2). Searching data for molecular weights that are similar to compounds with a retention of 1.25 obtained 3 compounds (NIST Chemistry WebBook SRD 69). The most similar compound based on molecular weight is erythromycin (C37H67NO13).

3. Retention Compound 1.606: According to the data in the graph below, compounds having a retention of 1.606 generate fractionation. Table 2 shows the molecular weight of the molecule with a retention of 1.606 is 1158.18. The NIST Chemistry WebBook SRD 69 contains no search results for a molecular weight similar to a molecule with a retention of 5.89. This is thought to be a novel chemical. Appendix 3 shows the index and relative intensity.

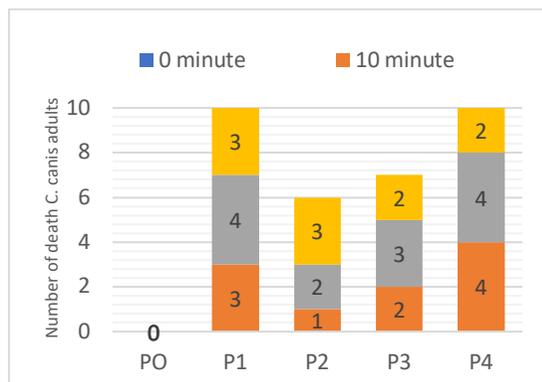
4. Retention Compound 4.299: As is evident in the graph below, compounds having a retention of 4.299 generate fractionation. The molecular weight of the molecule with a retention of 4.299 is 1148.56 (Table 2). The NIST Chemistry WebBook SRD 69 contains no search results for a molecular weight similar to a molecule with a retention of 4.299. This is believed to be a novel chemical. Appendix 4 provides the relative index and intensity of the retention compound 4.299.

5. Retention Compound 5.450: As demonstrated in the graph below, compounds having a retention of 5.450 generate fractionation. Table 2 shows the molecular weight of the molecule with a retention of 5.450 is 1150.17. The NIST Chemistry WebBook SRD 69 does not include any search data for a molecular weight similar to a molecule with a retention of 5.450. It is believed to be a novel chemical. Appendix 5 presents the relative index and intensity of the retention compound 8.59.

6. Retention Compound 7.784: As demonstrated in the graph below, compounds having a retention of 7.784 generate

fractionation. Table 2 shows the molecular weight of the molecule with a retention of 7.784 is 793.00. A search for molecular weight data on the NIST Chemistry WebBook SRD 69 obtained two similar compounds, namely Aluminum palmitate (C48H93AlO6) with a molecular weight of 793.20 and henpentacontylbenzene (C57H108) with a molecular weight of 793.46.

7. Ectoparasiticide Bioassay: A total of 10 adult *C. canis* individuals were tested. At minute zero, all treatments and controls had no death of *C. canis*. In the 10th minute, the highest mortality was shown in P4 and P1 positive control. In the 20th minute, half of the test animal population had died, as shown in the P3, P4 and control P1 treatments. In the P2 and P3 treatments, only eight individual *C. canis* died after 30 minutes of treatment. However, in the P4 and P1 treatments, all *C. canis* died (Fig. 2).



**Fig. 2.** The trend of *C. canis* mortality after combination extract treatment compared to control.

A total of 10 adult *C. felis* individuals were tested. At minute zero, all treatments and controls had no death of *C. felis*. At 10 and 20 minutes, the highest mortality was shown in P1, four individuals, and P4, three individuals. In the P2 and P3 treatments, only 6 and 7 individual *C. felis* died after 30 minutes of treatment. However, in the P4 and P1 treatments, all *C. felis* died (4).

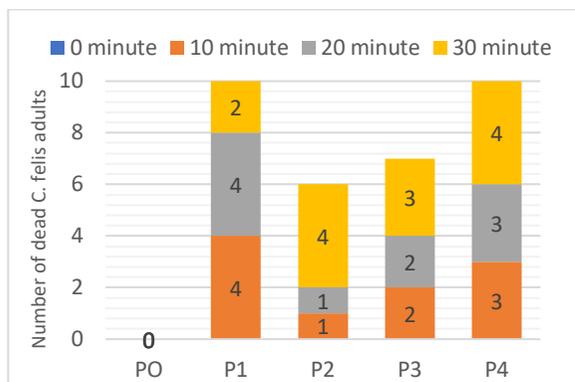


Fig. 3. The trend of *C. felis* mortality after combination extract treatment compared to control.

### 3.3 Analysis of Variance

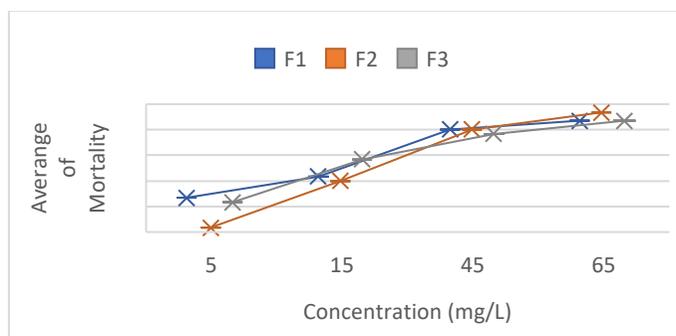
Analysis of variance of the five treatment combinations of extracts on *C. canis* showed a significant difference where F-count was 1.887 with a significance of 0.165 ( $\alpha = 0.05$ ). In the DMRT follow-up test, only P1 showed differences between treatments with P0, P2, P3 and P4 (appendix 2). Analysis of variance of the five treatment combinations of extracts on *C. felis* and *C. canis* showed a

significant difference where the F-count was 0.694 with a significance of 0.607 ( $\alpha = 0.05$ ). Duncan's test found no significant differences between treatments ( $\alpha = 0.05$ ) between P0, P1, P2, P3 and P4.

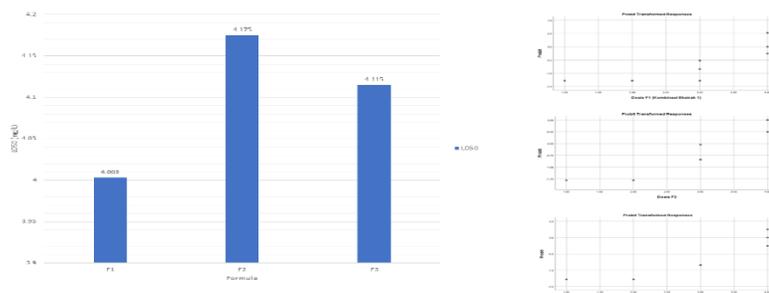
### 3.4 Lethal Dose 50 (LD50)

#### 3.4.1 Imago *C. felis*

The highest *C. felis* mortality after exposure to combined extract solutions was shown at a test concentration of 65 mg/L for all formulas. The lowest mortality rate was shown at the test concentration of 5 mg/L for all formulas. The formula with the highest average mortality is shown in F2, which was 9.33 (Fig. 4). Mortality data was used to determine the LD<sub>50</sub> using probit analysis with the SPSS program. The results of the probit analysis showed the highest toxicity in F1 (LD<sub>50</sub>=4.003 mg/L) and the lowest toxicity in F2 (LD<sub>50</sub>=4.175 mg/L) (Fig. 4).



(a)



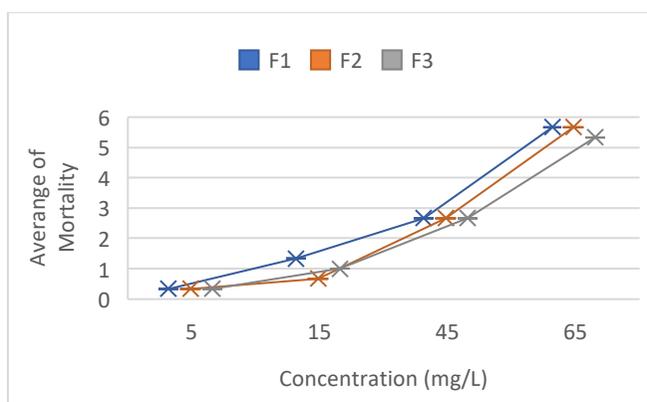
(b)

Fig. 4. (a). Mortality rate of combination extract formulations in *C. felis* (b). LD<sub>50</sub> extract against *C. felis*.

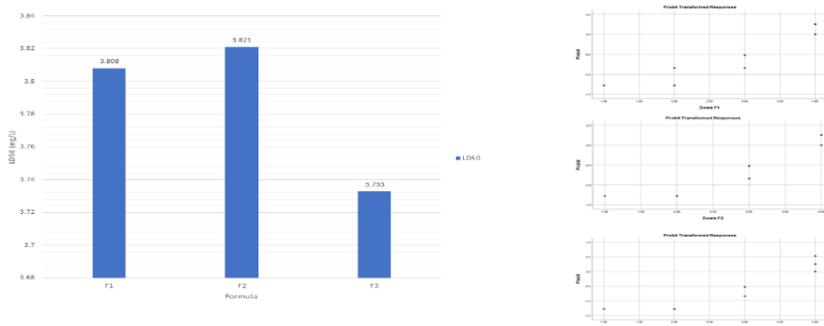
### 3.4.2 Imago *C. canis*

The highest *C. canis* mortality after exposure to combined extract solutions was shown at a test concentration of 65 mg/L for all formulas. All formulas had the lowest mortality rate at 5% test concentration. The combination of extracts with the highest average mortality is shown in F1, which is 8.33

(Fig. 5). Mortality data is used to determine the LD<sub>50</sub> using probit analysis in the SPSS program. The results of the probit analysis showed the highest toxicity in Formula 3 (LD<sub>50</sub>=3.733 mg/L) and the lowest toxicity in Formula 2 (LD<sub>50</sub>=3.823 mg/L) (Fig. 5).



(a)



(b)

**Fig. 5.** (a). Mortality rate of combination extract formulations in *C. canis* (b). LD<sub>50</sub> combination of F1, F2 and F3 extracts against *C. canis*.

Based on the mortality test, the combined extract formula showed a different level of toxicity in *C. felis* and *C. canis*. F2 showed the highest toxicity activity on *C. felis*, while F1 showed the highest toxicity on *C. canis*.

Phytochemical screening showed that Langusei extract contained alkaloids, flavonoids, saponins, and tannins at high intensity while triterpenoids and steroids were present at moderate intensity. Papaya latex is rich in proteases but includes peptides, other proteins, and the main phytochemical groups

[20, 21]. The phytochemical screening of the combination of Langusei ethanol extract and papaya latex showed high alkaloids, flavonoids, triterpenoids and steroids while saponins and tannins were present at moderate intensity. The content of proteases in papaya latex degrades phytochemical compounds that contain peptide and protein elements [22, 23]. This causes a change in the intensity of the content of the phytochemical group in the Langusei extract after being combined with papaya latex.

The combination of Langusei fruit extract and papaya latex was successfully analyzed using LC-MS/MS. The molecular weight, structure, identity, and quantity of individual sample components can be determined using LC-MS/MS data. Compounds are separated based on their respective interactions with the particle's chemical layer (stationary phase) and solvent elution via the column (mobile phase) [26]. The advantage of LC-MS is that it can evaluate a wider range of components, including thermally labile, high polarity, and high molecular mass chemicals, as well as proteins. The combination of ethanol extract from *Ficus minahassae* fruit and papaya latex produced five compounds based on LC-MS/MS analysis. Of the five compounds detected based on retention and molecular weight, only two compounds were successfully identified based on searching the online organic compound database. Thus, three compounds have not been reported, so they are stored in the NIST (USA) and AIST (Japan) databases. The first of the two compounds has a molecular weight of 1158.182 and a retention of 2.54. The second of the two compounds has a molecular weight of 880.61 and a retention of 8.29. These two compounds are thought to be novel compounds.

The ectoparasiticide test showed the highest average mortality in the P4 treatment for *C. canis* and *C. felis*. Based on the analysis of variance, the extract combination treatment affected the mortality of *C. canis* and *C. felis* ( $p < 0.05$ ). Even though the DMRT test did not show any differences between treatments, the compound content in the combination of extracts can have a toxic effect on *C. canis* and *C. felis*. Compounds detected in LC-MS play a role in the toxicity that causes death in *C. canis* and *C. felis*. The compounds were identified as 3-butenyl glucosinolate, TMS ((C26H59NO9S2Si5), erythromycin (C37H67NO13), Aluminium palmitate (C48H93AlO6) and henpentakontilbenzena (C57H108) menyebabkan iritasi [27-29].

The combination extract formulation 1 (F1) showed the highest toxicity with an LD<sub>50</sub> of 4.003 mg/L compared to F1 (4.175 mg/L) and F3 (4.115 mg/L) for testing on *C. felis*. The best LD<sub>50</sub> for *C. canis* is F3 (3.733 mg/L), then F1 (3.808 mg/L) and then F3 (3.821 mg/L). Thus, the LD<sub>50</sub> of the extract combination has a different effect on *C. canis* and *C. felis*. In *C. felis*, the composition of papaya latex, which was more present in the combination of extracts gave a strong toxic effect. In contrast, in *C. felis* the same ratio of *F. minahassae* extract and papaya latex showed a strong toxic effect. Cysteine protease in papaya latex is a natural protector against insect pests on papaya fruit [14, 30-32]. Papaya latex causes acute toxicity to *Aedes aegypti*, *Culex quinquefasciatus*, and *Sitophilus zeamais* [16, 33-35]. The proteolytic activity of papaya latex damages the cuticle of *Rhipicephalus microplus* as well [36].

Papaya latex contains proteases and secondary metabolite compounds such as alkaloids, terpenoids, proteins, phenols, and phytochemicals [15, 30]. Papaya latex is toxic to insects, mollusks, and fungi. The high LD<sub>50</sub> explains that the combination of *F. minahassae* fruit extract and papaya latex is synergistic in toxicity to the tested insects. As a Minahasa endemic plant, *Ficus minahassae* has little reporting on its use as an insecticide. The high content of phenolic compounds in *Ficus minahassae* fruit extract is consistent after being combined with papaya latex. Many phenolic compounds are reported to have insecticidal activity [36]. Allelochemicals from plants such as *Ficus sp*, *Ficus benghalensis*, and *Ficus religiosa* are insecticidal in a broad spectrum [37, 38]. *Ficus minahassae* is reported to have antibacterial activity as well [39]. The imago of *Ctenocephalides* sp. was exclusively examined in this study. Combinations of extracts should be tested on different life stages of *Ctenocephalides* in the immature phase in the future.

#### 4. Conclusion

The combination of *F. minahassae* extract with papaya latex produced five compounds with retention: 1.103, 1.258, 1.606, 4.299, 5.450, and 7.784  $\mu\text{m}$ , as determined by LCMS/MS. The five chemicals discovered were 3-butenyl glucosinolate, TMS (C<sub>26</sub>H<sub>59</sub>NO<sub>9</sub>S<sub>2</sub>Si<sub>5</sub>), erythromycin (C<sub>37</sub>H<sub>67</sub>NO<sub>13</sub>), aluminum palmitate (C<sub>48</sub>H<sub>93</sub>AlO<sub>6</sub>), and henpentacontylbenzene (C<sub>57</sub>H<sub>108</sub>). Some chemicals are thought to be novel. The combination extract had the highest toxicity for *C. felis* and *C. canis* in treatment P4 (10%), with an average mortality of 100%, the same as the synthetic pesticide control deltamethrin. Formula F1 produced the best LD<sub>50</sub> for *C. felis* (4.003 mg/L), while formula F3 produced the best LD<sub>50</sub> for *C. canis* (3.733 mg/L).

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