

Feeding Deterrent of Soft Coral Extracts *Nephthea* sp. Origin Sulawesi: A Developed Biopesticide Against *Plutella xylostella* Insect-Pest

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Abstract

Soft corals are known to produce metabolites with anti-predatory properties that could be developed into biopesticides with a feeding deterrent mechanism. Soft coral *Nephthea* sp. from Sulawesi waters has been reported to exhibit this activity. This study aims to evaluate the feeding deterrent activity of *Nephthea* sp. extract against the global pest *Plutella xylostella*, which has developed resistance to synthetic insecticides. Samples were extracted with MeOH:DCM (1:1) and fractionated into n-hexane, ethyl acetate, and ethanol fractions. Feeding deterrent activity was tested using the leaf disc method at concentrations of 50-250 mg/L, while toxicity was measured to determine LD₅₀. The results showed that all fractions significantly reduced larval feeding activity through an indirect mechanism. Probit analysis of LD₅₀ yielded values of 170.38 mg/L (EtOH), 128.54 mg/L (EtOAc), and 122.37 mg/L (n-hexane). GC-MS analysis of crude extracts identified 56 volatile and semi-volatile compounds, dominated by sesquiterpene derivatives. Computational toxicity prediction using ProTox 3.0 indicated that 62.53% of compounds had low oral toxicity or were non-toxic according to the Globally Harmonized System (GHS) classification. Although the LD₅₀ > 100 mg/L indicates moderate acute toxicity potential, the significant feeding deterrent activity at low concentrations (50-250 mg/L) and a promising computational safety profile suggest that *Nephthea* sp. has potential as a biopesticide that primarily acts through an feeding deterrent mechanism, but requires further formulation and toxicity studies before further development.

Keywords: Antifeedant, Anti-predatory, Green Mustard, Pro-Tox, Sesquiterpenoids

Introduction

Uncontrolled use of pesticides can result in environmental pollution and leave residues on agricultural products. Moreover, some farmers in Indonesia often use pesticides without regard to pest control needs, even when pests are not present. Using pesticides like this can lead to increased pesticide residues in agricultural products and environmental pollution (Lusiana & Rozi, 2015). According to the annual report of the Food and Drug Administration of the Republic of Indonesia (BPOM RI), in 2019, there were 334 cases of pesticide poisoning in Indonesia, of which 147 were caused by agricultural pesticides (Wijaya et al., 2022). Data from the Indonesian National Poison Information Center (SIKerNas) in 2017 reported that around 2.5% of poisoning incidents in Indonesia were caused by pesticides (Hasanah et al., 2022). Therefore, a safe alternative to pesticides from natural materials, known as biopesticides, is needed.

Biopesticides are pest-control substances derived from living organisms (plants, animals, or microorganisms)

that can inhibit the growth and development of pests or kill them (Daraban et al., 2023). Biopesticides control pests' growth, development, reproduction, and behavior (Utami et al., 2020). One mechanism of action of environmentally friendly biopesticides, namely their effect on pest feeding behavior, is their role as feeding deterrents (Pavela et al., 2025). The feeding-deterrent mechanism inhibits feeding without directly killing insects, while insects remain near the treated leaves and eventually die of starvation (Melanie et al., 2022).

Marine organisms are a promising and underexplored source of novel biopesticides. Their unique chemicals, evolved for defense and competition, often have biological activity against pests (Hanif et al., 2025; Hong et al., 2022). Marine biopesticides work by exerting feeding deterrence (Laborde et al., 2025), inhibiting growth (Chanthini et al., 2024), disrupting reproduction (Wang et al., 2025), or exerting direct toxicity (Beev et al., 2025). Among marine products, terpenoids—especially sesquiterpenoids and diterpenoids—are key for ecological defense and hold promise for pest

management due to their structural diversity and often lower persistence than synthetic analogues (Ninkuu et al., 2021; Qasim et al., 2024).

Soft corals are among the marine organisms of the class octocorallia that have been reported to produce compounds with anti-predatory properties (detering predators), serving as a chemical defense mechanism (Changyun et al., 2008; Scesa et al., 2022). This chemical defense allows soft corals to avoid predation despite lacking the rugged skeletal protection of stony corals (Nurrachma et al., 2021). Barre et al. (1986) reported that 50-100% of soft coral species exhibit chemical defense properties against predators. Previous studies have also reported sarcotrocheliol from the soft coral *Sarcophyton trocheliophorum* as feeding deterrent compounds (Yang et al., 2023). Soft corals of the family xeniidae have been reported to exhibit strong feeding-deterrent effects on fish assemblages (Yang et al., 2011). In addition, soft corals of the xeniidae family have been reported to produce antipredation substances as a chemical defense mechanism, which plays an important role in their abundance in Red Sea waters (Hoang et al., 2015).

The *Nephthea* genus (family nephtheidae) is rich in secondary metabolites, notably terpenoids, steroids, and prostaglandins (Allam et al., 2021). These compounds are part of the coral's chemical defenses, deterring predators (Abdelhafez et al., 2019). Notably, the sesquiterpene hydroxycolorone from *Nephthea chabrolii* shows feeding deterrent activity against *Spodoptera littoralis* larvae (Handayani et al., 1997). Literature supports testing *Nephthea* extracts as biopesticide candidates. However, the feeding deterrent activity of Sulawesi *Nephthea* sp. against *P. xylostella* has not been studied. This study aims to fill this gap by examining *Nephthea* sp. from this biodiverse region for potential new or potent bioactives.

This study explores the potential of soft corals, *Nephthea* sp., collected from the waters of Sulawesi, against the pest *P. xylostella*, which attacks cabbage, green mustard, and Brassica plants (Wicaksono et al., 2023). *Plutella xylostella* pest infestation can significantly reduce the yield and quality of cabbage and green mustard by feeding directly on the leaves, thereby affecting marketability and overall crop production (Bopape et al., 2014). The need for effective control of *P. xylostella* is urgent and globally recognized. It is one of the most destructive insect pests of cruciferous crops worldwide, causing estimated annual losses and control costs of US\$4-5 billion (Zalucki et al., 2012). Furthermore, its notorious ability to rapidly develop resistance to virtually all classes of synthetic insecticides, including *Bacillus thuringiensis* (Bt) toxins, has made it a "super-pest," rendering many conventional control strategies

ineffective (Furlong et al., 2013). This resistance crisis necessitates the development of alternative control agents with novel modes of action.

The literature review found no information on the feeding-deterrent activity of *Nephthea* sp. extracts collected from waters of Sulawesi against *P. xylostella*. The exploration of safe, environmentally friendly biopesticides is essential for Indonesia, where many farmers still do not prioritize food safety and environmental protection (Nanta et al., 2025). Therefore, it is necessary to develop safe, environmentally friendly biopesticides with feeding deterrent properties derived from natural products. In this study, a low concentration range (50-250 mg/L) was deliberately chosen to evaluate the potential of *Nephthea* sp. extract as a feeding deterrent against *P. xylostella*, with the aim of developing a natural and efficient biopesticide. This approach highlights the importance of demonstrating the extract's effectiveness at low concentrations for sustainable biopesticide development.

In this preliminary investigation, we used Gas Chromatography-Mass Spectrometry (GC-MS) as the primary dereplication tool to identify volatile and semi-volatile chemical components in crude extracts. This technique is highly effective for the initial identification of compound classes such as monoterpenoids and sesquiterpenoids, which are commonly found in soft corals and are known for their biological activity (Kasimala & Bollikolla, 2024). We recognize that GC-MS has limitations in detecting non-volatile, polar, or high-molecular-weight compounds. Therefore, the chemical profile obtained is considered a partial representation of the extract's complexity. The observed biological activity is hypothesized to arise from synergistic interactions between identified volatile compounds and other undetected non-volatile bioactive constituents detected by GC-MS. This holistic view still emphasizes the need for bioassay-guided fractionation using complementary techniques such as LC-MS/MS in future work to isolate the precise active principles.

Material and Methods

Materials

Methanol (MeOH), dichloromethane (DCM), n-hexane (n-hex), ethyl acetate (EtOAc), and ethanol (EtOH) of pro-analytical quality were obtained from MERCK for use as extraction solvents. Sodium hypochlorite (NaClO) solution was of technical grade. Dimethyl sulfoxide (DMSO) solution, used to dissolve the test extract, was also obtained from MERCK. *Plutella xylostella* larvae were collected from green mustard (*Brassica juncea* (L.) Czern.) plantations in Bayogé

Village, Palu City, Central Sulawesi. All experimental procedures were conducted at the Sekolah Tinggi Perikanan dan Kelautan Palu Laboratory.

Sampling and extraction

Samples of soft coral *Nephthea* sp. were collected from Palu Bay, Central Sulawesi, at coordinates 43.32 ° South latitude and 119.47 ° East longitude, at a depth of 3-5 m, in December 2016. Samples were transported to the laboratory in a cool container. Upon arrival, they were cut into 1×1 cm pieces and placed in glass containers. Several soft coral pieces were rinsed with a NaClO solution to facilitate observation of sclerites under a 100x microscope for genus-level identification. Genus-level identification was confirmed using colony morphology and sclerite characteristics, following the key of Fabricius & Alderslade (2001).

Then, 1000 g of soft coral samples were collected in a closed, light-proof bottle and macerated with a mixture of MeOH:DCM (v/v 1:1) (MERCK), using a sample-to-solvent ratio of 1:2 (w/v), while stirring using an orbital shaker (Joan Lab) for 20 hours (Putra et al., 2016). The crude extract was filtered and the solvent was evaporated under reduced pressure using a rotary vacuum evaporator (EYELA N-1100) to obtain a concentrated crude extract.

The concentrated crude extract was then subjected to sequential liquid-liquid partitioning to obtain primary fractions based on polarity (modified from Putra et al., 2016; Tanod et al., 2019). Briefly, the crude extract was first suspended in a 90% methanol-water solution (v/v). This aqueous methanolic suspension was then sequentially partitioned into three solvents of increasing polarity: n-hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar). For each partitioning step, the solvent was added at a 1:3 (v/v) ratio (suspended crude extract:solvent). The mixture was vigorously shaken in a separatory funnel and allowed to separate into two distinct layers. The organic layer was collected, and the process was repeated twice with fresh solvent to ensure exhaustive extraction. The remaining aqueous methanolic layer was then transferred to the next solvent in the sequence. The organic layers from each solvent (n-hexane, ethyl acetate, and ethanol) were collected separately and evaporated to dryness using a rotary evaporator, yielding the respective n-hexane fraction (n-Hex-F), ethyl acetate fraction (EtOAc-F), and ethanol fraction (EtOH-F). A simplified flowchart of the extraction and fractionation process is provided in Figure 1. The fractions were stored in a freezer prior to bioassay (feeding deterrent). Prior to assay, 25 mg of each n-hex, EtOAc, and EtOH extract was dissolved in 50 mL of DMSO 1%(MERCK). Serial

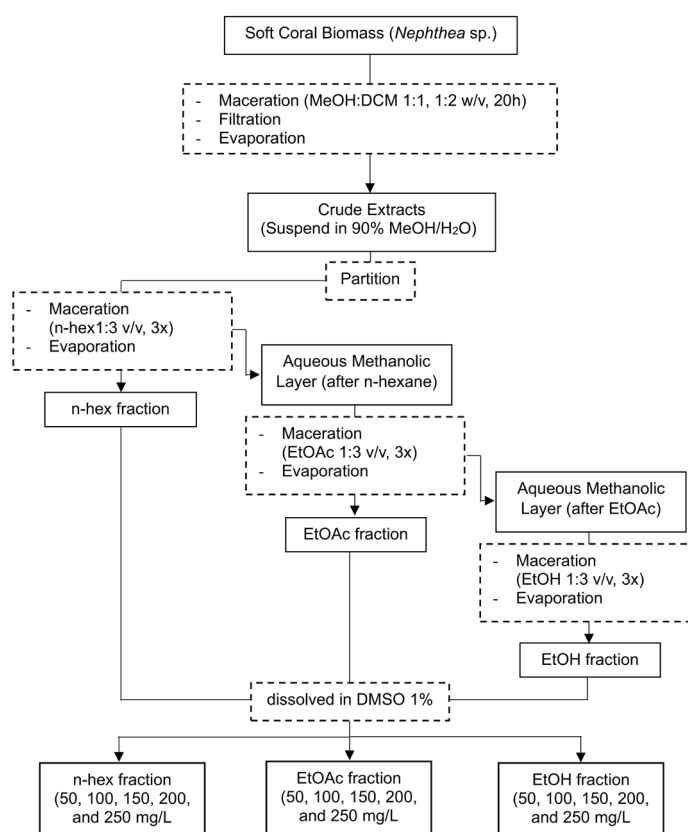


Figure 1. Soft coral extraction flow chart

dilutions were then prepared at concentrations of 50, 100, 150, 200, and 250 mg/L. DMSO 1% was used because it has been reported to show no toxic effects on larvae (Mickiewicz et al., 2025; Shabbir et al., 2021).

Preparation of test larvae

Third-instar *P. xylostella* pupae were obtained by rearing six-week-old green mustard plants that were not sprayed with synthetic pesticides (Komansilan et al., 2020). Breeding boxes measuring 5 × 1.5 m were made around the green mustard plants using nets. Larvae were prepared by collecting pupae from the green mustard farm, placing them in plastic bottles, and then hanging the bottles in the breeding box. At the top of the breeding box, a cotton swab was tied to a string and dipped in a honey-water solution (1:10). The pupae hatched and became moths after two days. The moth reproduces and lays its eggs on green mustard plants. The eggs hatch into instars I-III larvae. Furthermore, instar III larvae were used in the feeding deterrent activity assay (Figure 2). Prior to the assay, the larvae were not subjected to a starvation period. This decision was made to maintain the larvae in their normal physiological state and to better simulate natural field conditions, where larvae typically have continuous access to host plants. This approach helps avoid potential stress-induced feeding behaviors that might occur in starved larvae (Charabidze et al., 2013; Mohite et al., 2023).

Assaying of feeding deterrent activity

The assay was conducted using the leaf-disc method as described by Atta-ur-Rahman et al. (2001). In a sterile petri dish, wet filter paper is placed, and the surface is coated with a transparent, perforated plastic sheet. The leaf discs were made by sticking a 1-cm cork borer into green mustard leaves that had not been sprayed with synthetic pesticides. The leaf discs were the same size, shape, and thickness. Furthermore, leaf discs were dipped in EtOH, EtOAc, and n-hexane extracts (50, 100, 150, 200, and 250 mg/L) dissolved in 1% DMSO. This assay used comparative controls: Methomyl 40SP 2 mg/mL in

aquades (positive control) and, for the negative control, aquades and DMSO 1%. Green mustard leaf discs dipped in the extracts showed no physical changes, and their color remained the same as that of the leaf discs in the control treatment. The study was conducted with three repetitions. Leaf discs were dipped for 5 minutes and then aerated for 5 minutes. After aeration, the leaf discs were weighed and put into the prepared petri dishes. Each petri dish contains five *P. xylostella* larvae, which were meticulously observed for their response to the extract-treated leaf discs. These observations were made after 24 hours, and the nature of feeding deterrence was carefully noted. The parameter observed was the weight of the remaining leaves that the larvae had not eaten. Leaf discs were then weighed to determine the weight of green mustard leaves eaten by *P. xylostella* larvae. The feeding deterrent activity was measured by calculating the percentage of feeding reduction (% FR) using the formula of Pari et al. (1998):

$$\% \text{ Feeding Reduction} = \left\{ 1 - \frac{\text{weight of eaten leaves in the treatment group}}{\text{weight of eaten leaves in the control group}} \right\} \times 100$$

Toxicity measurement of extracts against test larvae

The toxicity measurements were performed using the method of Atta-ur-Rahman et al. (2001). Transparent glass bottles of 100 mL capacity were filled with EtOH, EtOAc, and n-hexane extracts (50, 100, 150, 200, 250 mg/L), positive (Methomyl 40SP, 2 mg/mL in distilled water), and negative (Aquades and DMSO, 1%) controls. The extract was evenly spread on the entire surface of the bottle, which was then aerated for 10 minutes. A total of 25 *P. xylostella* larvae were placed into the glass bottle and covered with gauze. Observations were made after 24 hours, and the number of larvae that experienced mortality was counted with thoroughness and attention to detail. Toxicity assaying was performed three times for each extract fraction. The toxicity level of the extract was measured by the percentage of mortality according to Abbott's formula (Heinrichs et al., 1981):



Figure 2. Third-instar larvae of *P. xylostella*

$$\% \text{ Mortality} = \left\{ \frac{\% \text{ mortality in the treatment group} - \% \text{ mortality in the control group}}{100 - \% \text{ mortality in the treatment group}} \right\} \times 100$$

Gas chromatography and mass spectrometry (GC-MS) analysis

GC-MS identified compounds in the crude extracts of soft coral *Nephthea* sp. The extract was diluted with methanol, filtered through a 10 µL sterile syringe filter, and 1 µL of the extract solution was injected into the gas chromatograph. GC-MS analysis was performed using a Hewlett-Packard (HP) 6890 gas chromatograph equipped with an electron ionization mass spectrometer (Hewlett-Packard, USA). A dimethylpolysiloxane column with 5% diphenyl (Elite 5MS, PerkinElmer, USA) was used for separation (30 m length, 250 µm diameter). High-purity helium was used as the carrier gas. Pneumatic injection was used to introduce the sample at a 50:1 split ratio. The oven temperature was initially set to 150°C, then increased to 240°C at 10°C/minute, and held at this maximum temperature for 22 minutes. The mass spectrometer inlet and source temperatures were maintained at 300°C. The scanning range was 50-550 amu. Structure determination was based on analyzing mass-spectral fragmentation patterns and directly comparing mass spectra with profiles in the National Institute of Standards and Technology (NIST) and Wiley Libraries (Tanod et al., 2019). The confidence level of compound identification with GC-MS, performed by comparing mass spectrum fragmentation patterns and retention times with those in the NIST and Wiley libraries, can be seen in the 'Quality' value (%). Given the known limitations of these libraries for marine natural products, identification is considered tentative, with Quality values above 70% indicating a higher level of confidence (Wei et al., 2014). Gas Chromatography-Mass Spectrometry (GC-MS) is an instrument that can quantify the amount of each compound identified in a natural extract (Sami, 2022). GC-MS analysis can serve as an initial screening of the volatile and semi-volatile chemical composition of a natural product, which can help determine the potential benefits of natural extracts (El-Naggar et al., 2023; Gomathi et al., 2015). It should be noted that the GC-MS analysis of the crude extract of *Nephthea* sp. used in this study was optimized for the identification of volatile and semi-volatile compounds, especially those representing monoterpenoids and sesquiterpenoids.

Toxicity class prediction

Predictions were made of compounds from *Nephthea* sp. soft coral extracts, which GC-MS detected. Toxicity prediction was done using Pro-Tox 3.0 - prediction of toxicity of chemicals (<https://tox.charite.de/protox3/>

[index.php?site=compound_input](index.php?site=compound_input)) using canonical SMILES from <https://pubchem.ncbi.nlm.nih.gov/> (Banerjee et al., 2024). Furthermore, compounds identified by GC-MS were predicted to have aquatic toxicity rules, nonbiodegradable properties, and Ames toxicity using ADMETlab 3.0 (<https://admetlab3.scbdd.com/server/evaluation>) (Fu et al., 2024).

Data analysis

The effect of different solvent types and concentrations on feeding reduction and mortality was analyzed by ANOVA, and if $F_{\text{count}} > F_{\text{Table}}$, followed by Tukey test. Mortality data were used for Lethal Dose (LD₅₀) determination and analyzed using the probit method. ANOVA and probit analysis were performed using SPSS 20.0.

Results and Discussion

Soft coral samples were identified based on colony shape, polyps, and surface sclerites using an identification key from the Australian Institute of Marine Science (Fabricius & Alderslade, 2001). The soft coral samples were identified as *Nephthea* sp. based on distinct morphological features and sclerite structures (Figure 3).

The fractionated extracts of *Nephthea* sp. were assayed for feeding deterrent properties by observing the percentage of feeding reduction of green mustard leaves against *P. xylostella* larvae. The results of the feeding deterrent activity assay are shown in Figure 4.

Figure 4 shows that the higher the extract concentration, the greater the extract's ability to reduce the feeding activity of *P. xylostella* larvae. In the negative control group, green mustard leaf discs were soaked in water and 1% DMSO; after 24 hours, the discs were completely consumed by *P. xylostella* larvae. This indicates that the solvent used to dissolve the extracts (DMSO 1%) had no effect on the feeding activity of *P. xylostella* larvae. At 250 mg/L, EtOAc extracts showed the highest feeding reduction, and 50 mg/L EtOH extracts showed the lowest. Compared with the positive control of commercial pesticides with 2 mg/mL of methomyl 40SP, the ability of EtOH, EtOAc, and n-hexane extracts of *Nephthea* sp. 50 mg/L showed better feeding reduction. Methomyl is a carbamate insecticide commonly used to control pests by inhibiting appetite. However, according to the U.S. Environmental Protection Agency (EPA) and the World Health Organization (WHO), methomyl is classified as highly toxic to the environment and humans (Frentinia, 2010; Yuan et al., 2022).

The mechanism of action of methomyl is as a stomach poison that kills pests when ingested (Hidayati

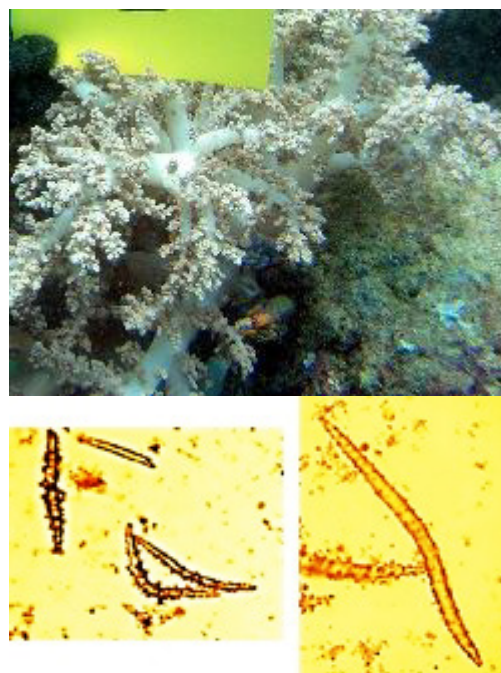


Figure 3. Morphology and sclerites of *Nephthea* sp. origin Sulawesi

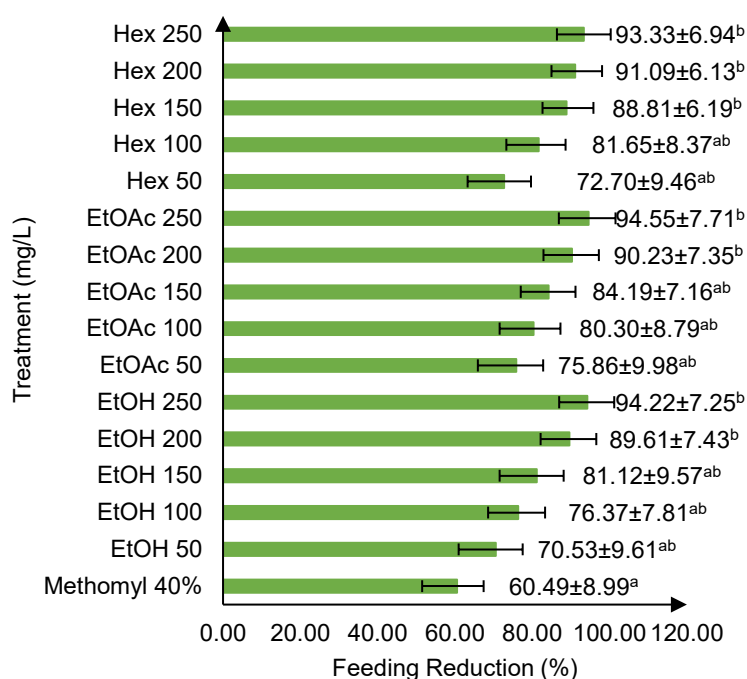


Figure 4. Feeding reduction of *Nephthea* sp. extracts on feeding activity of *P. xylostella* larvae

& Ciptono, 2022). ANOVA results show a significant effect of solvent type and the concentration of the extract provided. Tukey test results show that the ability of 40% methomyl feeding reduction is almost the same as EtOH (50, 100, 150 mg/L), EtOAc (50, 100, 150 mg/L), and n-hexane (50, 100 mg/L). At the same time, the ability of EtOH extract (200 and 250 mg/L) is almost equivalent to EtOAc (200 and 250 mg/L) and n-hexane (150,

200, 250 mg/L). It should be noted that this significant feeding deterrent activity was achieved at relatively low concentrations (50-250 mg/L). The effectiveness at this concentration range indicates the high potential of bioactive compounds in *Nephthea* sp. extracts. The ability to disrupt pest feeding behavior at low concentrations is a highly desirable attribute in the development of biopesticides, as it not only reflects the efficacy of the

active compound but also promises a better safety profile and cost efficiency (Ayilara et al., 2023; Hamouche et al., 2024).

Feeding deterrent substances can stop or inhibit the feeding activity of herbivores or predators (Yooboon et al., 2021). The mechanism of action of feeding deterrents can work directly or indirectly, quickly or slowly (Liu et al., 2020). Feeding deterrent substances that work directly can immediately stop the feeding activity of insect pests (Singh et al., 2021). In contrast, those that work indirectly show the effect after the feeding deterrent substance is digested (Singh et al., 2025). The results showed that the soft coral extracts of *Nephthea* sp. worked indirectly because there were many parts of the green mustard leaves that were eaten, so it was thought that the *P. xylostella* larvae stopped their feeding activity after digesting the *Nephthea* sp. extract first.

After that, toxicity testing of *Nephthea* sp. extracts against *P. xylostella* larvae was conducted. This toxicity assay was conducted to obtain the LD₅₀ of *Nephthea* sp. extract using probit analysis. The toxicity assay of *Nephthea* sp. extracts on the mortality of *P. xylostella* larvae is shown in Figure 5.

Figure 5 shows that the higher the extract concentration, the higher the mortality of *P. xylostella* larvae. In the negative control group (aquades and 1% DMSO), after 24 hours, no *P. xylostella* larvae died. These results indicate that the solvent used to dissolve the extracts (DMSO 1%) is not toxic to *P. xylostella* larvae. The toxicity of the extracts showed that EtOH (200 and 250 mg/L), EtOAc (150, 200, 250 mg/L), and n-hexane (150, 200, 250 mg/L) treatments had >50% mortality. In contrast, the EtOH (50, 100, 150 mg/L), EtOAc (50 and

100 mg/L), and n-hexane (50 and 100 mg/L) treatments had mortality <50%. In the treatment of n-hexane extract, 250 mg/L showed the highest mortality, and EtOH extract 50 mg/L showed the lowest mortality. Research by Salaki et al. (2012) also showed that the nonpolar fraction showed high mortality of *P. xylostella* larvae.

The probit analysis determined the LD₅₀ values, which are the concentrations estimated to cause 50% mortality in the test population, as follows: EtOH extract (LD₅₀ 170.38 mg/L), EtOAc extract (LD₅₀ 128.54 mg/L), and n-hexane extract (LD₅₀ 122.37 mg/L). The determination of LD₅₀ in biopesticide development aims to obtain biopesticides that show maximum effects (in this study, the feeding deterrent effect) in pest control and minimal impact on the environment and non-target organisms (low toxic) (Nurmianti & Gusmarwani, 2020; Punjungsari, 2022).

The results of the probit analysis show LD₅₀ values for all fractions above 100 mg/L, indicating an acute oral toxicity profile in the moderate-low category, when compared to highly potent synthetic neurotoxic insecticides such as methomyl (Könemann et al., 2022). These test results suggest that the main strength of *Nephthea* sp. extracts may not lie in their rapid lethal action, but rather in their significant feeding inhibition activity observed at low concentrations (50-250 mg/L). For biopesticide candidates designed to work primarily as feeding deterrents, the main criteria are their effectiveness in suppressing feeding activity and their safety profile (Wang et al., 2025). Significant feeding reduction (up to 80% at higher concentrations within the tested range) combined with low mammalian and environmental toxicity predicted computationally for most of its semi-volatile and volatile constituents, places this extract

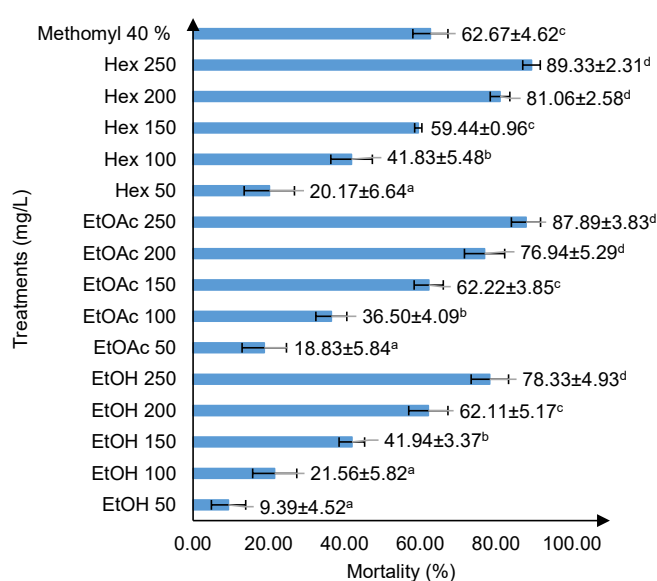


Figure 5. Toxicity of *Nephthea* sp. extract on mortality of *P. xylostella* larvae

as a promising candidate for further development (Pavela et al., 2025). Further research will focus on improving effectiveness through formulation, isolating specific feeding deterrent compounds, and conducting comprehensive toxicity testing on non-target organisms.

Previous studies have shown that soft coral metabolite compounds, derivatives of terpenoid and steroid groups, are widely soluble in semi- to non-polar fractions (Allam et al., 2021). The EtOH extract of soft coral *Sinularia* sp. from Sulawesi waters has been confirmed as an feeding deterrent (Tanod et al., 2018). Chloromertensine compounds from soft corals have demonstrated antifeedant and antifouling properties (Fotouh Selim, 2012). Soft corals, in their natural environment, produce feeding deterrent substances to deter reef fish from preying on their eggs (Hoang et al., 2015), highlighting the intricate ecological interactions. In a seminal study, it was reported that *Nephthea chabrolii* produces hydroxycolorone compounds that exhibit strong feeding deterrent activity against larvae of the insect *Spodoptera littoralis* (Handayani et al., 1997).

Crude extract of soft coral *Nephthea* sp., macerated with MeOH:DCM, was investigated using a GC-MS instrument. GC-MS analysis of the crude extract successfully identified 56 volatile and semi-volatile compounds, most of which were sesquiterpene derivatives (Table 1; some data have been published previously, Tanod et al. (2019)). Table 1 identifies 56 compounds detected in 64 RT, and 37 compounds in 41 RT are detected with a quality level $\geq 70\%$. Although sesquiterpenoids and other compounds identified by GC-MS (Table 1) have also been reported to originate from terrestrial sources, the prevalence of these compounds in this extract is highly consistent with the chemical profile established for the genus *Nephthea* (Allam et al., 2021; Amir et al., 2012). The identifications presented are tentative, as is common in the GC-MS dereplication process; however, the high 'Quality' scores (many above 70%) for the major compounds provide additional confidence in these tentative assignments. Overall, this chemical profile strongly supports the idea that the observed feeding deterrent activity is associated with a sesquiterpenoid-rich chemical defense mechanism characteristic of soft corals.

It is widely known that sesquiterpenoids exhibit strong feeding deterrent activity (Evelyn et al., 2024; Riyadi et al., 2023). However, the strong feeding inhibition activity of this crude extract is not thought to be entirely due to the volatile compounds detected by GC-MS. We suspect a synergistic effect, whereby the identified volatile sesquiterpenoid compounds interact with non-volatile compounds, such as diterpenoids with feeding deterrent activity, which have been reported in soft coral extracts (Elkhawas et al., 2020; Yan et al., 2023). Literature studies have also reported volatile sesquiterpenoid derivatives

from soft corals (Yu et al., 2024; Zhu et al., 2024). Therefore, the overall biological activity results from the combination of the direct deterrent effect of volatile compounds and the post-digestive effect of non-volatile compounds (Allam et al., 2023; Cheng et al., 2009).

Derivatives of sesquiterpenoid compounds have been reported in preliminary studies, showing promising feeding deterrent properties. For instance, sesquiterpene compounds from the bark of *Cinnamosma* sp. exhibited significant feeding deterrent activity against adult female mosquitoes (Inocente et al., 2019). Similarly, sesquiterpenoids isolated from *Artemisia vestita* demonstrated moderate feeding deterrent activity against third-instar larvae of *P. xylostella*, with LD₅₀ values ranging from 25.3 to 42.1 $\mu\text{g}/\text{cm}^2$ (Ding et al., 2019). Two sesquiterpenes isolated from *Senecio palmensis* also showed feeding deterrent properties against *Leptinotarsa decemlineata* larvae (Gonzalez-Coloma et al., 1995). The mechanistic role of sesquiterpene compounds as feeding deterrents involves their role as TRPA1 (Transient Receptor Potential Ankyrin 1) channel agonists, which leads to an excitatory response in sensory neurons. This activation is linked to nociceptive signaling, which is crucial for detecting noxious substances and triggering avoidance behavior in animals (Escalera et al., 2008). Furthermore, the mechanism of sesquiterpene compounds as TRPA1 channel agonists also stimulates reduced insect feeding behavior responses (Salgado, 2017). Although reports on feeding deterrent sesquiterpenoids from soft corals (particularly *Nephthea* sp.) remain very limited, the literature has reported chemical defense activity in several species of soft corals. Sesquiterpenoids from *Alcyonium grandis* have been reported to be feeding deterrents against marine predators (sea stars) (Carbone et al., 2009). Asteriscane-type sesquiterpenoids from *Sinularia capillosa* have been reported to inhibit larval attachment (Chen et al., 2013). Liang & Guo (2013) have reported that terpenoids from the genus *Sarcophyton* exhibit feeding deterrent activity. The potential of sesquiterpenoid compound derivatives as candidates for developing pest control strategies is a significant and promising outcome of this research, with potential implications for the field of pest control.

Figure 5 also shows significant loss of the n-hexane and ethyl acetate fractions, indicating the presence of non-volatile bioactive compounds that were not detected by the GC-MS analysis method. This suggests that the overall toxicity and effectiveness of the *Nephthea* sp. extract stems from the combined effects of its volatile and non-volatile components. The toxicity predictions presented are based exclusively on the profile of volatile and semi-volatile compounds identified through GC-MS analysis. The current toxicity prediction approach for crude extracts of *Nephthea* sp. does not account for non-volatile constituents, particularly high-molecular-weight

Table 1. GC-MS evaluation of *Nephthea* sp. crude extracts origin Sulawesi

RT (min)	Compounds	Area (%)	Quality	Group
3.385	delta-Elementene	16.63	98	Sesquiterpene
3.596	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	0.45	87	Monoterpene
3.836	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)- [1S-(1.alpha.,2.beta.,4.beta.)]-	1.3	90	Sesquiterpene
4.128	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR (1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	0.22	92	Sesquiterpene
4.185	(-)-Aristolene	0.68	99	Sesquiterpene
4.419	2-Hydroxy-7-methylquinoline-3-carbaldehyde	0.29	47	Quinoline
4.471	Isoledene	0.51	95	Sesquiterpene
4.676	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	1.54	93	Sesquiterpene
4.711	Neoisolongifolene, 8,9-dehydro-	0.22	90	Sesquiterpene
5.259		0.85	92	
4.745	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	0.55	90	Sesquiterpene
4.791	.delta.-Selinene	1.13	99	Sesquiterpene
4.888	.gamma.-Elementene	0.52	94	Sesquiterpene
5.002	(3S,4R,5S,6R,7S)-aristol-9-en-3-ol	0.31	83	Sesquiterpene
6.814		0.69	70	
7.968		0.35	41	
5.557	Selin-4,7(11)-diene	0.25	92	Sesquiterpene
5.734	Isospathulenol	1.94	64	Sesquiterpene
7.437		0.42	90	
5.837	Isolongifolan-8-ol	0.25	48	Sesquiterpene
5.882	6,11-Guaiadien-4-ol	0.78	64	Sesquiterpene
5.957	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)-	0.53	93	Sesquiterpene
6.031	Dehydroaromadendrene	1.37	58	Sesquiterpene
6.111		0.72	70	
6.454		0.5	70	
6.162	Selina-6-en-4-ol	0.33	91	Sesquiterpene
6.237	4,8,8-Trimethylspiro[2.6]non-4,6-diene	12.09	55	Sesquiterpene
6.334	1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	4.72	90	Monoterpene
6.551	.Alpha.-guaiene	0.5	70	Sesquiterpene
6.883	1-[2'-ethenyl-1'-cyclohexenyl]-2-methyl-2-propen-1-one	1.09	59	Flavonoid
6.997	Tridecanoic acid, 12-methyl-, methyl ester	0.88	97	Fatty acid
7.18	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1.81	91	Monoterpene
7.225	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	0.56	91	Monoterpene
7.34	Isolongifolene, 9,10-dehydro-	0.66	90	Sesquiterpene
7.563	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	3.4	59	Aromatic hydrocarbon
7.614	1H-Cycloprop[e]azulene-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	0.41	93	Sesquiterpene
7.757	Alloaromadendrane-4beta,10alpha-diol	5.52	52	Sesquiterpene
7.848		9.54	53	

Table 1. GC-MS evaluation of *Nephthea* sp. crude extraxts origin Sulawesi (cont.)

RT (min)	Compounds	Area (%)	Quality	Group
8.111	Allo-Aromadendrene	0.22	56	Sesquiterpene
8.471	Germacrone	0.7	47	Sesquiterpene
8.591	Isoaromadendrene epoxide	0.29	86	Sesquiterpene
8.683	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methyl-ethyl)-	0.47	49	Cycloalkane
8.746	Benzenamine, 2,4,6-trimethyl-	3.79	35	Aromatic amine
9.06	Hexadecanoic acid, methyl ester	1.22	97	Fatty acid
9.226	.beta.-caryophyllene	0.34	92	Sesquiterpene
9.294	3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	0.93	49	Cycloalkene
9.409	n-Hexadecanoic acid	1.03	96	Fatty acid
10.003	Oxirane, hexadecyl-	0.82	94	Alkyl epoxide
10.117	Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)] dodecane, 3,3,6,6,9,9,12,12-octamethyl-, anti,anti,anti-	0.31	62	Polycyclic hydrocarbon
10.163	1,10-dimethyl-2-methylene-trans-decalin	1.75	42	Cycloalkene
10.54	Benzenemethanamine, 4-methoxy-	0.75	38	Aromatic amine
10.609	4-Hexadecen-6-yne, (Z)-	0.58	68	Alkyne
10.632	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, (4aS-cis)-	0.37	50	Alkyne
10.752	9-Octadecenoic acid, methyl ester,(E)-	2.38	99	Fatty acid
10.969	Octadecanoic acid, methyl ester	0.52	97	Fatty acid
11.106	Octadec-9-enoic acid	0.97	99	Fatty acid
11.306	1-Hexadecene	0.47	80	Alkene
11.346	Thiosulfuric acid, S-(2-aminoethyl) ester	0.37	47	Thiosulfonic acid
11.409	Methyl 3-methoxytetradecanoate	0.36	45	Fatty acid
12.386	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	3.31	94	Fatty acid
12.472	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	0.81	95	Fatty acid
14.17	Tetrapentacontane, 1,54-dibromo-	0.41	91	Dibrominated alkane
14.398	Eicosane	0.45	92	Alkane
18.267		1.65	91	

diterpenoids characteristic of soft corals. Consequently, this computational toxicity prediction should be regarded as a preliminary assessment of a limited portion of the chemical space of the crude extract of *Nephthea* sp.

Furthermore, 56 compounds detected by GC-MS were computationally predicted for LD₅₀ and toxicity class and classified based on The Globally Harmonized System of Classification and Labelling of Chemicals (GHS). LD₅₀ in units (mg/kg), namely Class I: fatal if swallowed (LD₅₀≤5); Class II: fatal if swallowed (5<LD₅₀≤50); Class III: toxic if swallowed (50<LD₅₀≤300); Class IV: harmful if swallowed (300<LD₅₀≤2000); Class V: may be harmful if swallowed (2000<LD₅₀≤5000); and Class VI: non-toxic (LD₅₀>5000) (Atolani et al., 2024). The LD₅₀ prediction results and toxicity classes of the *Nephthea* sp.

crude extract compounds detected by GC-MS are shown in Figure 6.

Figure 6 shows that 1.79% of the compounds belonged to Class II, Class III 10.71%, Class IV 23.21%, Class V 55.36%, and Class VI 8.93%. The toxicity class prediction results indicated that 87.50% of the compounds detected from the crude extracts of *Nephthea* sp. were non-toxic to harmful if swallowed interval. Furthermore, the target toxicity was predicted based on the guidelines of Banerjee et al. (2018) into hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity (Table 2). It is crucial to emphasize that the toxicity predictions presented in Table 2 and Figure 6 are based solely on the profiles of volatile and semi-volatile compounds identified by GC-MS analysis. This approach

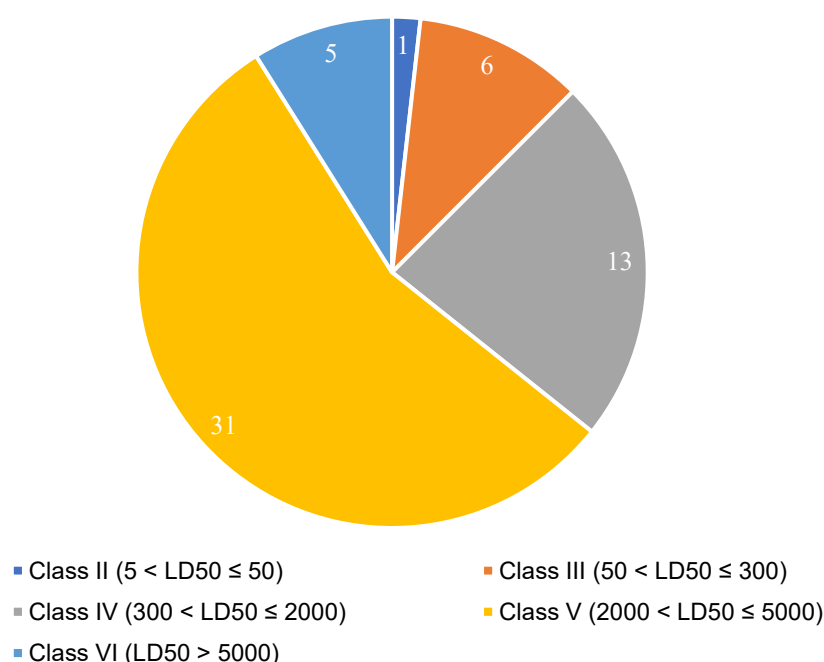


Figure 6. Predicted LD50 and toxicity class of *Nephthea* sp. crude extract compounds detected by GC-MS

does not account for the non-volatile constituents, which are often the dominant and most bioactive compounds in soft corals, such as high-molecular-weight diterpenoids, steroids, and polyhydroxylated compounds (Amir et al., 2012; Liang & Guo, 2013). Consequently, this computational toxicity prediction should be regarded as a preliminary and conservative assessment of a limited portion of the chemical space of the crude extract of *Nephthea* sp. A comprehensive safety profile would require isolating major non-volatile components and evaluating them individually for toxicity.

Analysis of the target toxicity predictions in Table 2 shows that two compounds are predicted to be neurotoxic with probability > 0.7. While 19 compounds are predicted to be neurotoxicity, 1 compound is predicted to be nephrotoxicity, seven compounds are predicted to be respiratory toxicity, and 1 compound is predicted to be cardiotoxicity (with probability value < 0.7). Suppose the probability value is close to 1 in the toxicity prediction analysis using ProTox 3.0; this indicates that the prediction model has a very high confidence level in the predicted toxicity results. In addition, a probability value close to 1 also indicates that the tested compounds are highly similar to the compounds in the training dataset used to build the ProTox 3.0 prediction model (Banerjee et al., 2024). The results of the target toxicity prediction analysis in Table 2 show that, in general, the compounds of *Nephthea* sp. crude extract evaluated by GC-MS are inactive against hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity.

In addition to the predicted toxicity to human target organs, the compounds in the *Nephthea* sp. crude extract evaluated by GC-MS were also predicted for their effects on the aquatic environment (aquatic toxicity rule), non-biodegradability, and mutagenic potential (Ames Toxicity). The aquatic toxicity rule prediction results indicated that only the allo-aromadendrene compound (0.22%) was predicted to be toxic to aquatic environments. The aquatic toxicity rule prediction results are supported by the non-biodegradability prediction results. The prediction results showed that 48 of 56 compounds detected by GC-MS were predicted to be biodegradable, offering optimism about their environmental impact. Ames toxicity predictions showed 43 compounds (69.22%) with probability values < 0.400, 12 compounds (29.75%) with probability values between 0.401 - 0.700, and 1 compound (0.81%) with probability values > 0.701. The Ames toxicity prediction results showed that the compounds detected from the crude extract of *Nephthea* sp. by GC-MS were not mutagenic.

Aquatic toxicity rule and non-biodegradable in ADMETlab 3.0 are toxicity prediction features that help identify compounds that can potentially cause toxicity to aquatic organisms (Dong et al., 2018). Prediction of aquatic toxicity and non-biodegradable is important because pesticide residues usually flow into waters, affecting organisms and the aquatic environment (Aryani & Wahyuningsih, 2021). Ames Toxicity prediction in ADMETlab 3.0 aims to identify whether a compound is potentially mutagenic. Positive results from the Ames test

indicate that the compound is likely mutagenic and could potentially be a carcinogen (Nursanti et al., 2023). The results of the analysis that has been carried out prove that the extract of soft coral *Nephthea* sp. has the potential to be developed as a biopesticide material that is safe for humans and the environment, with a feeding deterrent effect.

Beyond the demonstrated bioactivity as feeding deterrent, the sustainability of *Nephthea* sp. as a biopesticide source warrants consideration. *Nephthea* species are distributed across tropical coral reefs and can exhibit considerable local abundance in Indonesian waters (Putra et al., 2022; Wirabuana et al., 2019). While not currently utilized in commercial biopesticide production, their potential application aligns with the growing interest in marine-derived agrochemicals (Marrone, 2019). Importantly, recent advances in soft coral aquaculture through mariculture and fragment propagation suggest viable pathways for sustainable biomass production (Leal et al., 2016; Nguyen et al., 2022). Furthermore, the relatively high extraction yield

of bioactive compounds from small amounts of biomass enhances the economic feasibility of utilizing this resource. These factors, combined with the potent feeding deterrent activity demonstrated in this study, position *Nephthea* sp. as a promising and sustainable candidate for further biopesticide development.

It is important to note a limitation of this study: the GC-MS analysis was performed on the crude extract to provide an initial chemical profile, while the bioassay-active fractions (n-hexane, ethyl acetate, and ethanol) were not individually subjected to GC-MS due to limited sample quantity after extensive biological testing. This presents a clear and compelling direction for future research. A subsequent, larger-scale investigation should focus on the bioassay-guided fractionation of the most active extracts. This approach will allow for the detailed chemical characterization of each sub-fraction using techniques like GC-MS and LC-MS/MS, ultimately leading to the isolation and identification of the specific compounds responsible for the potent feeding deterrent activity observed in this study.

Table 2. Prediction of target toxicity from *Nephthea* sp. crude extracts based on GC-MS prediction

Compounds	Prediction of Target Toxicity																			
	Hepatotoxicity				Neurotoxicity				Nephrotoxicity				Respiratory Toxicity				Cardiotoxicity			
	<0.7		>0.7		<0.7		>0.7		<0.7		>0.7		<0.7		>0.7		<0.7		>0.7	
	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I
Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-																				
Cyclohexene, 3-methyl-6-(1-methylethylidene)-																				
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1.alpha.,2.beta.,4.beta.)]-																				
1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR (1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-																				
(-)-Aristolene																				
2-Hydroxy-7-methylquinoline-3-carbaldehyde																				
Isoledene																				
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-																				
Neoisolongifolene, 8,9-dehydro-																				

Compounds	Prediction of Target Toxicity															
	Hepatotoxicity				Neurotoxicity				Nephrotoxicity				Respiratory Toxicity			
	<0.7		>0.7		<0.7		>0.7		<0.7		>0.7		<0.7		>0.7	
	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I
Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-																
.delta.-Selinene																
.gamma.-Elemene																
(3S,4R,5S,6R,7S)-aristol-9-en-3-ol																
Selin-4,7(11)-diene																
Isospathulenol																
Isolongifolan-8-ol																
6,11-Guaiadien-4-ol																
Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)-																
Dehydroaromadendrene																
Selina-6-en-4-ol																
4,8,8-Trimethylspiro[2.6]non-4,6-diene																
1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene																
.Alpha.-guaiene																
1-[2'-ethenyl-1'-cyclohexenyl]-2-methyl-2-propen-1-one																
Tridecanoic acid, 12-methyl-, methyl ester																
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol																
7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-																
Isolongifolene, 9,10-dehydro-																
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-																
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-																
Alloaromadendrane-4beta,10alpha-diol																
Allo-Aromadendrene																
Germacrone																
Isoaromadendrene epoxide																

Compounds	Prediction of Target Toxicity																			
	Hepatotoxicity				Neurotoxicity				Nephrotoxicity				Respiratory Toxicity				Cardiotoxicity			
	<0.7		>0.7		<0.7		>0.7		<0.7		>0.7		<0.7		>0.7		<0.7		>0.7	
	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I
Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-																				
Benzenamine, 2,4,6-trimethyl-																				
Hexadecanoic acid, methyl ester																				
.beta.-caryophyllene																				
3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-																				
n-Hexadecanoic acid																				
Oxirane, hexadecyl-																				
Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)] dodecane, 3,3,6,6,9,9,12,12-octamethyl-, anti,anti,anti-																				
1,10-dimethyl-2-methylene-trans-decalin																				
Benzenemethanamine, 4-methoxy-																				
4-Hexadecen-6-yne, (Z)-																				
1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, (4aS-cis)-																				
9-Octadecenoic acid, methyl ester,(E)-																				
Octadecanoic acid, methyl ester																				
Octadec-9-enoic acid																				
1-Hexadecene																				
Thiosulfuric acid, S-(2-aminoethyl) ester																				
Methyl 3-methoxytetradecanoate																				
5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-																				
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-																				
Tetrapentacontane, 1,54-dibromo-																				
Eicosane																				
Legend:	A	Active																		
	I	Inactive																		

Conclusion

This research has uncovered that the soft coral extract *Nephthea* sp. from Sulawesi exhibits strong feeding deterrent properties at low concentrations (50-250 mg/L) against *P. xylostella*, an insect pest, with a suspected indirect mechanism of action. Although its toxicity test results ($LD_{50} > 120$ mg/L) fall into the moderate-low category, the main potential of this extract lies in its ability to effectively disrupt pest feeding behavior. The favorable toxicity profile of the *Nephthea* sp. extract, supported by computational predictions of its preliminary safety profile, indicates its potential as a biopesticide candidate that operates through a behavior-modification mechanism. Further research will prioritize the isolation of the active feeding deterrent principle through bioassays, the development of formulations to improve durability and effectiveness, and rigorous toxicity evaluations on non-target species to fully assess its environmental safety for agricultural applications.

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