Angeles City Science High School

Lourdes Sur East, Angeles City

**Research Title:**

# "Bioactivity of Finger-Leaf Morning Glory Plant (*Ipomoea digitata)* Extract against Asian Subterranean termite (*Coptotermes gestroi)*"

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# Introduction

Termites are known for being highly destructive to mainly wood products, wooden furnitures and other wooden-related materials.

The negative effects of termites can be disturbing and too destructive to many different plants and trees. Termites are mainly known for damage caused to human beings, both in rural and urban areas (Figueirêdo et al., 2015). In the United States of America, they cause more economic damage than fire and flood combined, predominantly by feeding on structural timbers (Bignell et al., 2010). A small percentage of the community understands the purpose of termites in improving soil quality but many people don't acknowledge this rather they treat termites as pest. However, people in urban areas don't want the termites to destroy the wooden furnitures that they use. It is estimated that approximately 600,000 homes are damaged by termites in the US each year; $5 billion dollars are spent annually by US residents to control termites and repair damage; and atleast $1 billion dollars are spent on Fermosan termite control and repairs each year, although some experts estimate it is closer to $2 billion (Northwest Exterminating, 2019). The global damage caused by termites was estimated at US $22 billion to US $40 billion worldwide (Su, 2002; Rust and Su, 2012) and in Southeast Asia alone, it was estimated to cost approximately US $ 400 million per year (Lee, 2007). Furthermore, the subterranean termite cause economic losses was estimated that $ 32 billion in 2010 worldwide for control and damage

repairs (Rust and Su, 2012). Subterranean termites attack accounted for 90% of the total economic loss and about 70% of damage of construction.

*Coptotermes gestroi*, commonly known as Asian subterranean termite, are native to Southeast Asia which has been introduced to China, the Americas, Italy and some Pacific islands (Aool, 2014). About 90% of property damages in the Philippines are caused by Coptotermes species making it the "public enemy number 1" in the Philippines. One of the most commonly found termite species in the Philippines is the *C. gestroi* (Rentokill, 2020).

The following research had been conducted that was demonstrated from mangrove leaf extract (*Rhizophora species)* that states extractives or flavonoids present in these extract could be toxic or unfavorable for termites, where the ethyl acetate extract was responsible for the greater resistance of heartwood in teak wood. Some flavonoids, such as quercetin and taxifolin might be useful for termite control agents because they are abundant in plants (Syahidah & Subekti, 2019). However, a study about the anti-termite properties of *Protium javanicum* Burm. f. extract states the following. Scopoletin, quercetin, and stigmasterol were isolated by bioassay-guided fractionation. Scopoletin had the highest activity among the three compounds (Adfa et al., 2010). In conclusion, scopoletin has higher activity than the quercetin that is present in the mangrove leaf extract (*Rhizophora sp.*).

*Ipomoea digitata,* commonly known as finger-leaf morning glory plant, the two compounds from an experiment were isolated from ethanol extract of *Ipomoea digitata*. The compounds are Scopoletin (7-hydroxy-6-methoxycoumarin) and β-sitosterol glucoside (Mofiz et al., 2015). This plant is commonly found in East and Southeast Asia and is commonly used as a herbal plant. To summarize, *I. digitata* contains high content of scopoletin.

The issues about the current termiticides have significant side effects. An example of this is the most popular Taurus SC, a termiticide, the results take some time and you can only use it on outside. The active ingredient it contains is Fipronil (This Old House Reviews Team, 2021). Researchers found thyroid tumors in both male and female rats fed the highest dose. While these findings are considered to apply only to rats, fipronil is classified as a "possible human carcinogen" by the United States Environmental Protection Agency (U.S. EPA). Scientists found that fipronil is highly toxic to sea and freshwater fish, and highly toxic to sea and freshwater invertebrates (National Pesticide Information Center, 2009). In conclusion, chemical termiticides are harmless to our human body but it is harmful to our environment.

The purpose of this study is to determine and experiment the anti-termite (termiticidal and anti-feedant) activity of finger-leaf morning glory plant (*I. digitata)* against Asian subterranean termite (*C. gestroi).* This is done by extracting one of the parts of finger-leaf morning glory plant by using alcohol extracts.

This study seeks to find a potential solution to kill local termites (*C. gestroi)* using a flavanoid from an extract of the finger-leaf morning glory plant (*I. digitata).* Termites are considered to be one of the most destructive pest and people spend so much money to try and eliminate it in their homes. If no mass-produced and easily renewable source of termiticides without any significant side-effects are found, then people will keep spending too much money and will result to the economy collapsing.

This study seeks to answer the following questions:

- Does it have any high anti-termite activities?

- Which solvent is the best for achieving the highest termiticidal and anti-feedant activity in the experiment?
- How long does the anti-termite effect last?
- By how much is the effectiveness of the best extract against termites?

This study will assess if the finger-leaf morning glory plant extract can be a viable alternative to the current termiticides.

The following hypotheses were formed for this study:

*Ho:* If the finger-leaf morning glory plant (*I. digitata*) extract does not have any anti-termite effects or activities against *C. gestroi* then there is no need to study further.

*Ha:* If the finger-leaf morning glory plant (*I. digitata)* does have anti-termite effects or activities against *C. gestroi* then it might become an alternative or replace the current chemical termiticides.

This study is significant to our environment and economy. As mentioned above, the global damage caused by termites was estimated at US $22 billion to US $40 billion worldwide (Su, 2002; Rust and Su, 2012). This could help us grow our economy by creating the natural termiticides and save money from expensive chemical termiticides. This could also save our environment from destruction of woods. This study will serve as a guide for future researchers about this topic.

## Methodology

### 1. Materials

The materials that will be used in this study by the researcher are the following; 100g of leaves of Finger-leaf morning glory plant (*I. digitata)*, 10-20 Asian subterranean termites (*C. gestroi)*, 10-20 paper disc, food-grade oil filters and laboratory equipments such as rotavapor. The leaves of finger-leaf morning glory plant will be collected from Bulacan, Philippines. The researcher can identify and described the plant by its following characteristics. It is a large, smooth, perennial climber rising from stout, somewhat fleshy roots. Leaves are on long petioles, rounded in outline, and heart-shaped at the base. Lobes are 5 to 7, usually lanceolate, 5 to 15 centimeters long, often reaching nearly to the base. Flowers are 3 to 5, on a stalk in the leaf axil. Sepals are ovate, concave, and 1 centimeter long. Corolla is pink, purple, broadly bell-shaped, smooth and about 6 centimeters long. Capsules are ovoid, 1 centimeter long or less (Stuart, Jr., 2016) .

The Asian subterranean termites (*C. gestroi)* will be obtained from a nearby mould in the local area. The paper disc and the food grade oil filters will be purchase from an online store. The following materials should be in the laboratory where the experiment will be conducted. The laboratory must have 125mL Erlenmayer Flasks, Amber glass vials or amber vial. The following alcohols should be in the laboratory, if not then the researcher will purchase it from an online store; undenatured 70% ethanol to extract the flavonoids; Methanol, N-Butanol, and Ethyl Acetate (≥ 98.0% Analytical Reagent grade standards) (Lv et al., 2020).

## 2. Research Locale

The researcher will contact the Philippine Institute of Pure and Applied Chemistry (PIPAC) for a possible facility or laboratory for the experiment that will be conducted. The laboratory must have a Laboratory freezer, Rotavapor, Laboratory Heating Bath, and the vapor. The laboratory must also have the laboratory equipments mentioned in the Materials section.

## 3. Procedure

### 3.1. Preparation of plant materials or Maceration

The leaves will be dried in the oven at the temperature of 60 °C and ground into powder form using a mortar and pestle and sieved into average particle size of 215µm. The powdered sample will be kept in freezer to prevent the growth of microorganisms and protect the sample from light prior to extraction. (Lee et al., 2016).

### 3.2 Extraction of leaves by macerated process

5.0 g of dried powder leaves of Finger-leaf morning glory plant will be place into macerated in 75 mL of distilled water and place into heat bath at constant speed of 220 rpm and retention time of 30 minutes. The shaking maceration process will be perform at 25 °C. Meanwhile, shaking maceration process was also done at a retention time of 30 minutes at constant temperature of 75 °C with constant speed of 220 rpm. (Lee et al., 2016). Repeat the process for all the samples but with different solvents.

### 3.3. Termite Bioassay

No-choice and two-choice bioassay will be employed to assess the termiticidal activity of finger-leaf morning glory plant leaves. The weight loss of the paper discs will be used to determine the termiticidal properties of extract that will obtain by the following equation: in the no-choice bioassay, the absolute coefficient of antifeedancy (A) = [(KK – EE)/(KK + EE)] x 100(%), while in the two-choice bioassay, the relative coefficient of antifeedancy (R) = [(K – E)/(K + E)] x 100(%); where KK (K) and EE (E) are the weight losses of the control and treated paper discs, respectively. The total coefficient of antifeedancy (T) is equal to A plus R. All extracts tested will be classified into the following classes according to their T values; feeding preference ($T < 0$), class I ($0 \leq T < 50$), class II ($50 \leq T < 100$), class III ($100 \leq T < 150$), class IV ($150 \leq T < 200$) and 200 for complete antifeedant (Syahidah & Subekti, 2019).

## 4. Data Analysis

The table shown below is the data collected from different extracts.

**Table 1.** Weight loss of paper disc in no-choice bioassay.

| No. of Trials | Solvents | Weight Loss paper disc (%) |
|---|---|---|
| 1 | N-Butanol (n-BuOH) | |
| | Ethanol | |
| | Ethyl Acetate (EtOAc) | |
| | Methanol | |
| | Control Group | |
| | N-Butanol (n-BuOH) | |

| | | |
|---|---|---|
| 2 | Ethanol | |
| | Ethyl Acetate (EtOAc) | |
| | Methanol | |
| | Control Group | |
| 3 | N-Butanol (n-BuOH) | |
| | Ethanol | |
| | Ethyl Acetate (EtOAc) | |
| | Methanol | |
| | Control Group | |

**Table 2.** Weight loss of paper disc in two-choice bioassay.

| No. of Trials | Solvents | Weight Loss paper disc (%) |
|---|---|---|
| 1 | N-Butanol (n-BuOH) | |
| | Ethanol | |
| | Ethyl Acetate (EtOAc) | |
| | Methanol | |
| | Control Group | |
| 2 | N-Butanol (n-BuOH) | |
| | Ethanol | |
| | Ethyl Acetate (EtOAc) | |

| | | |
|---|---|---|
| | Methanol | |
| | Control Group | |
| 3 | N-Butanol (n-BuOH) | |
| | Ethanol | |
| | Ethyl Acetate (EtOAc) | |
| | Methanol | |
| | Control Group | |

**5. Statistical analysis**

As a result of multiple analysis of variation (ANOVA) tests, the type of extracts of all the samples was evaluated their anti termite and antifungal activity. A software was utilized as a statistical tool. For Post Hoc analyses, a Scheffe test will be use to compare values at a level of significance of $p < 0.05$ (Syahidah & Subekti, 2019).

**6. Risk and Safety**

Before proceeding to the experiment, the researcher must follow the General Laboratory Safety Rules. Avoid using mobile phones and eating inside the laboratory. The researcher must have an accompanied professional to supervise the researcher. The researcher must wear gloves, laboratory coat, laboratory glasses or goggles, proper laboratory boots. The containers should be labeled appropriately. Use the appropriate temperatures when using the Heat bath, Laboratory Freezer, and other laboratory equipments.

**7. Disposal**

The containers must be properly sanitized after doing the experiment. The excess leaves from Finger-leaf morning glory plant can be store for a future experiment. The excess alcohol concentrations ethanol should be kept in a safe place with regular room temperature. The oil filters should be disposed properly by washing it with distilled water then it must be disposed in a waste container.

## References

Govorushko, S. (2018). Economic and ecological importance of termites: A global review. *Wiley Online Library.* https://onlinelibrary.wiley.com/doi/pdf/10.1111/ens.12328

Figueirêdo, R. E. C. R. D., Vasconcellos, A., Policarpo, I. S., & Alves, R. R. N. (2015). Edible and medicinal termites: a global overview. *Journal of Ethnobiology and Ethnomedicine*, *11*(1). https://doi.org/10.1186/s13002-015-0016-4

Bignell, D. E., Roisin, Y., & Lo, N. (2010). *Biology of Termites: a Modern Synthesis* (2nd ed. 2011 ed.). Springer. https://books.google.com.ph/books?hl=en&lr=&id=8yvE5lg8reoC&oi=fnd&pg=PR7&dq=termites&ots=j52WKMj6Zq&sig=z9ED9mDFtWF2bk5wFljEoHBkUPs&redir_esc=y#v=onepage&q=termites&f=false

Northwest Exterminating. (2019). *How Much Damage Can Termites Really Cause? | Termite Control*. https://www.callnorthwest.com/2019/02/how-much-damage-can-termites-really-cause/

Evans, T. A. (2019). *Not just urban: The Formosan subterranean termite, Coptotermes formosanus, is invading forests in the Southeastern USA*. Biological Invasions. https://link.springer.com/article/10.1007/s10530-018-1899-5?error=cookies_not_supported&code=3afca93f-9a31-48b5-aadc-d8e098fb47e7

Kuswanto, E., Ahmad, I., & Dungani, R. (2015). *Threat of Subterranean Termites Attack in the Asian Countries and their Control: A Review*. Science Alert. https://scialert.net/fulltext/?doi=ajaps.2015.227.239#223956_ja

Su, N.Y. (2002). Novel technologies for subterranean termite control. *Sociobiology*, 40: 95-102.

Rust, M. K., & Su, N. Y. (2012). Managing Social Insects of Urban Importance. *Annual Review of Entomology*, *57*(1), 355–375. https://doi.org/10.1146/annurev-ento-120710-100634

Lee, C.Y. (2007). *Perspective in Urban Insect Pest Management in Malaysia.* Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Malaysia, ISBN-13: 9789833986071, Pages: 104.

Aool, W. (2014). *Coptotermes gestroi (Asian subterranean termite)*. Cabi. https://www.cabi.org/isc/datasheet/15286

Rentokill. (2020). *Termite Species*. https://www.rentokil.com/ph/termites/termite-species/

Syahidah, & Subekti, N. (2019). *Biological Activity of Mangrove Leaves Extract (Rhizophora sp.)*. IOPscience. https://iopscience.iop.org/article/10.1088/1755-1315/270/1/012051

Adfa, M., Yoshimura, T., Komura, K., & Koketsu, M. (2010). Antitermite Activities of Coumarin Derivatives and Scopoletin from Protium javanicum Burm. f. *Journal of Chemical Ecology*, *36*(7), 720–726. https://doi.org/10.1007/s10886-010-9807-1

Mofiz, N., Hossain, M. D., & Khan, U. (2015). *Scopoletin and β-sitosterol glucoside from roots of Ipomoea digitata*. ResearchGate.

https://www.researchgate.net/publication/283070675_Scopoletin_and_b-sitosterol_glucoside_from_roots_of_Ipomoea_digitata

This Old House Reviews Team. (2021). *The Top 5 Termite Killers of 2021*. This Old House. https://www.thisoldhouse.com/pest-control/21238556/best-termite-killers

National Pesticide Information Center. (2009). *Fipronil General Fact Sheet*. NPIC. http://npic.orst.edu/factsheets/fipronil.html

Stuart, Jr., G. (2016). *Aurorang-gubat, Ipomoea digitata Linn., WILD YAM ROOT : Philippine Medicinal Herbs / Alternative Medicine*. StuartXchange. http://www.stuartxchange.org/AurorangGubat.html

Lv, Z., Luo, Z., Lu, J., Xu, Z., Zhang, W., & Chen, A. (2020). Analysis of metabolites of nitrofuran antibiotics in animal-derived food by UPLC-MS/MS. *International Food Research Journal*, 467–478. http://ifrj.upm.edu.my/28%20(03)%202021/06%20-%20IFRJ20102.R1.pdf

Lee, N. Y., Yunus, M. A. C., Idham, Z., Ruslan, M. S. H., Aziz, A. H. A., & Irwansyah, N. (2016). Extraction and identification of bioactive compounds from agarwood leaves. *IOP Conference Series: Materials Science and Engineering*, *162*(1). https://doi.org/10.1088/1757-899x/162/1/012028