

OPTIMIZATION OF SOXHLET EXTRACTION OF *IPOMOEALBA* L. AND EVALUATION OF A BOTANICAL PESTICIDE FORMULATION

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Abstract:

Ipomoea alba L. (Convolvulaceae) is a vigorously growing wild plant with potential as a source of botanical pesticides. This study optimized Soxhlet extraction of *I. alba* and evaluated a pesticide formulation containing its extract. Extraction time (150 - 300 minutes), plant material-to-solvent ratio (1:20 - 1:50, w/v), and ethanol concentration (50 - 96.5%, v/v) were examined. The highest extraction yield, 35%, was obtained after 240 min at a material-to-solvent ratio of 1:30 using 70% ethanol. A formulation containing neem leaf extract (3%), *I. alba* extract (7.5%), and niaouli oil residue (4.5%) produced 80% mortality within 3 hours in a spray assay. In a food-dipping assay, mortality reached 70% after 24 hours and 80% after 36 hours.

Keywords: *Ipomoea alba* L., Soxhlet, bio-pesticide, antibacterial, formulation

Tối ưu hóa quá trình chiết Soxhlet đối với cây bìm bìm một thuỳ, hoa trắng (*Ipomoea alba* L.) để ứng dụng điều chế thuốc trừ sâu sinh học

Tóm tắt:

Bìm bìm một thuỳ, hoa trắng (*Ipomoea alba* L.) thuộc họ Convolvulaceae là loại cây mọc hoang, sinh trưởng mạnh và có tiềm năng làm nguyên liệu sản xuất thuốc bảo vệ thực vật có nguồn gốc thực vật. Nghiên cứu này tối ưu hóa quá trình chiết xuất *Ipomoea alba* L. bằng phương pháp Soxhlet và đánh giá hiệu quả của chế phẩm chứa dịch chiết từ loài cây này. Các yếu tố được khảo sát gồm thời gian chiết (150 - 300 phút), tỷ lệ nguyên liệu trên dung môi (1:20 - 1:50, khối lượng/thể tích) và nồng độ ethanol (50 - 96,5%, thể tích/thể tích). Hiệu suất chiết cao nhất, đạt 35%, thu được sau 240 phút với tỷ lệ nguyên liệu trên dung môi là 1:30 và nồng độ ethanol 70%. Chế phẩm gồm dịch chiết lá neem (3%), dịch chiết **I. alba** (7,5%) và phần cặn dầu trầm (4,5%) cho tỷ lệ chết 80% sau 3 giờ trong thử nghiệm phun. Trong thử nghiệm nhúng thức ăn, tỷ lệ chết đạt 70% sau 24 giờ và 80% sau 36 giờ.

Từ khóa: Bìm bìm, Soxhlet, thuốc trừ sâu, cặn dầu trầm năm gân, công thức

1. Introduction

Ipomoea alba L. (syn. *Calonyction aculeatum* (L.); Convolvulaceae) is an annual climbing plant, widely cultivated as an ornamental species for its fragrant, nocturnally blooming white flowers (Ono et al., 2026). In Vietnam, this species grows abundantly in the wild, particularly on damp, open ground, along riverbanks, and in hedgerows in suburban or rural areas. Its invasive nature has been reported (Castañeda-Gómez et al., 2017).

Over the past two decades, global use of synthetic pesticides has increased rapidly to meet the agricultural production demands of a growing world population. However, the widespread application of these synthetic chemicals is a major cause of environmental pollution and poses serious risks to public health, while also accelerating the development of pesticide resistance in harmful insects. Consequently, plant-based pest management products are attracting significant interest as eco-friendly, biodegradable, and highly selective alternatives that minimize toxicity to non-target organisms (Kamaraj et al., 2010; Kamaraj et al., 2023; Silvério et al., 2020). Over the last half-century, numerous plant species have been screened in the search for insecticidal and insect-repellent agents (B.S. Kumar, 2022; Chatterjee et al., 2023; Mukesh Sharma, 2023). In Vietnam, extensive research has also been conducted on naturally derived pesticides. Researchers-including Vu Quang Con et al., Nguyen Duy Trang, Quach Thi Ngo, Nguyen Truong Thanh, Nguyen Thi Quynh, Tran Dinh Pha, and Bui Lan An-have pioneered the development of botanical pesticides from native flora, demonstrating promising field efficacy ranging from 48% to over 90% (Bùi Lan Anh, 2018).

Currently, commercial products derived from neem leaf extracts are diverse and widely available on the market. Neem leaf extracts are rich in azadirachtin-a potent tetranortriterpenoid compound with a broad spectrum of activity; it functions by repelling insects, inhibiting oviposition, and disrupting hormonal regulation and feeding behaviors (Gever et al., 2026). Numerous studies have confirmed the insecticidal efficacy of neem leaf extracts in minimizing damage and controlling pest populations across various crops (such as tomatoes and *vigna radiata*) through mechanisms that prevent egg-laying and cause direct mortality (Chatterjee et al., 2023; Fernandes et al., 2019; Gevers et al., 2026).

To evaluate and develop new raw materials and formulations for pest control, a study titled " Evaluation of Soxhlet Extraction Parameters of *Ipomoea alba* L. for Bio-Pesticide Application " was conducted. This research aimed to optimize the Soxhlet extraction process for *Ipomoea alba* L.. The resulting extract was combined with neem leaf extract and niaouli oil residue. The ultimate goal was to utilize a wild plant species (*Ipomoea alba* L.) in conjunction with agricultural by-products (niaouli oil residue) and neem leaf extract to develop a highly effective, multi-component biopesticide formulation.

2. Methodology

2.1. Material and chemicals

Ipomoea alba L. was collected from a garden in Vung Tau Ward, Ho Chi Minh City (Vietnam). The leaves were sorted and washed to remove yellow or substandard leaves. The material was then dried in an convection dryer at 60°C for 5–6 hours until the moisture content dropped below 5%. The dried material was ground into a powder (particle size ≤ 80 mesh) using an FWJ-04 grinder (China). After processing, the powder was stored in PP zip-lock bags at room temperature. The moisture content of the powder was determined to be 4.85% using an MB45 moisture analyzer (Ohaus, China).

Neem leaf extract (NE) and niaouli oil residue were provided by the Faculty of Applied Science and Technology at Nguyen Tat Thanh University. The niaouli oil residue was recovered from the bottom of the distillation column during the molecular distillation of crude essential oil, which was conducted to isolate 1,8-cineole as the target fraction.

2.2. Methods

2.2.1. Conventional extraction by Soxhlet

A 15.0 g sample of the powder was placed in a 6.0 × 10.0 cm filter bag. The packaged sample was loaded into the extraction chamber of a Soxhlet apparatus, while ethanol solvent was charged into the boiling flask. The experimental variables evaluated included extraction time (min), solid-to-liquid ratio (w/v), and ethanol concentration (%). Experiments were conducted according to the design parameters presented in Table 1. Following extraction, the solvent was removed from the extract using vacuum rotary evaporation (Heidolph, Germany). Finally, the resulting extract was weighed and stored in an amber glass vial at room temperature for subsequent analysis.

Table 1. The levels of operating parameters

Parameters	Levels
Extraction time (min)	150 - 180 - 210 - 240 - 270 - 300
Solid-to-solvent ratio (w/v)	1:20 - 1:30 - 1:40 - 1:50
Ethanol concentration (%)	50 - 70 - 80 - 96.5

Source: Author's calculation

2.2.2. Preparation of insecticide formulation

In the initial stage, the oil residue was mixed with Tween 40 and subjected to homogenization for 30 minutes to form the dispersed phase. Concurrently, extracts from *Ipomoea alba* L. (IE) and neem leaves (NE) were dissolved in a 20% (v/v) ethanol solution. This solution was then heated to 70°C to completely remove the ethanol; the resulting solution was referred to as the continuous phase. The continuous phase was cooled to 40°C and subjected to continuous mechanical stirring. The dispersed phase was slowly added to the continuous phase, and the mixture was subsequently homogenized for 30 minutes using a high-speed homogenizer. The composition and ingredient ratios for each formulation are presented in Table 2.

Table 2. Ingredient composition for formulation efficacy evaluation

Formulation	Content (w/w %)		
	<i>Ipomoea alba</i> L. extract	Neem leaf extract	Niaouli oil residue
1	6	3	6
2	6	4.5	4.5
3	6	6	3
4	7.5	3	4.5
5	7.5	4.5	3
6	7.5	6	1.5
7	9	3	3
8	9	4.5	1.45
9	9	6	0

Source: Author's calculation

2.2.3. Yield of extract

The percentage yield was obtained using dry weight:

$$\text{Yield of extract (\%)} = \frac{W_1}{W_2} \cdot 100$$

Where W_1 (g) is the weight of the extract residue after solvent removal and W_2 (g) is the weight of dried plant powder.

2.2.4. In Vitro insecticidal efficacy of spray-based bioassays

A spray-based bioassay was conducted using mealworms (20-30 mm in length). Two milliliters of the prepared solution for each test formulation were evenly sprayed onto vegetable leaves (serving as the food source); The samples were then transferred to Petri dishes. Subsequently, mealworms were placed onto the treated plant material. The Petri dishes were covered with fine mesh cloth to prevent the larvae from escaping while ensuring adequate ventilation. Larval mortality was monitored at specific intervals post-exposure: 30 minutes, 120 minutes, and 150 minutes.

This bioassay evaluated various formulations-using distilled water and 30% (v/v) ethanol as control groups-following a Completely Randomized Design (CRD) with three replicates. Corrected insecticidal efficacy was calculated using Abbott's formula:

$$H(\%) = \frac{C - T}{C} \cdot 100 \quad (1)$$

Where:

- C is the number of surviving Mealworms in the control group;
- T is the number of surviving Mealworms in the treated group;

2.2.5. In Vitro Insecticidal Efficacy via Leaf-Dip Bioassay

A leaf-dip bioassay was conducted using mealworms (20–30 mm in length). Fresh vegetable leaves were washed with distilled water, air-dried at room temperature, and cut into circular discs with a diameter of 1 cm. To evaluate each pesticide formulation, a total of ten leaf discs were prepared: five were fully immersed in the test solution, removed with forceps, and allowed to air-dry for 20–30 minutes; the other five were immersed in distilled water as a control, following the same procedure.

To maintain leaf moisture, a 1.5% (w/v) agar solution was poured into Petri dishes to a thickness of 1 mm and allowed to solidify at room temperature. The prepared leaf discs were then arranged in the Petri dishes in an alternating pattern, comprising five dishes containing treated samples and five containing water-treated controls. Ten mealworms were introduced into each Petri dish. Mealworm mortality and behavior were monitored at 24 and 36 hours post-exposure. The bioassay was conducted in the laboratory using a Completely Randomized Design (CRD) with three replicates. Insecticidal efficacy was calculated using Abbott's formula.

3. Results and Discussion

3.1. Effect of Soxhlet extraction parameters on high recovery yield

3.1.1. Effect of the period of time on the yield of the extract

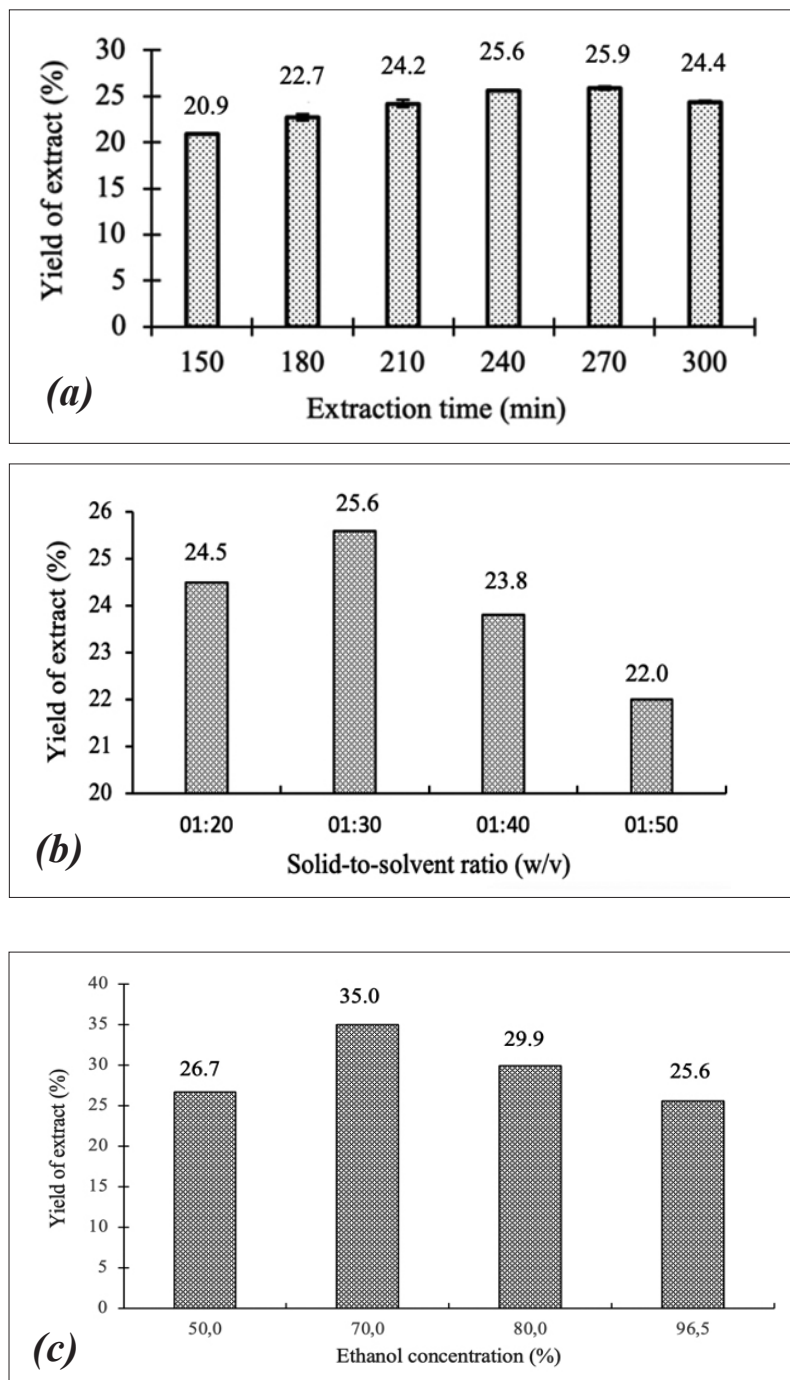
Extraction time significantly affects extraction yield (Suwari et al., 2017, 2018). Figure 1(a) illustrates the effect of extraction time on yield, with fixed parameters: solid-to-liquid ratio (1:30) and ethanol concentration (96.5%). Within the investigated range of 150 to 300 minutes, the yield peaked at 25.9% at 270 minutes before gradually declining. The rapid extraction rate during the initial stage is attributed to the high solubility of surface compounds and a strong mass transfer driving force (Gong et al., 2012). However,

excessively long extraction times lead to the denaturation and degradation of bioactive compounds due to overheating, thereby reducing extraction yield (Alara et al., 2018). Although the maximum yield (25.9%) was achieved at 270 minutes, an extraction time of 240 minutes-yielding a comparable 25.6%-was selected for subsequent studies. This choice is justified because the potential increase in yield is negligible, whereas the shorter operating time results in significant savings in both time and energy consumption.

3.1.2. Effect of solid-to-solvent ratio on the amount of extract

The solid-to-solvent ratio is another critical parameter; generally, increasing the solvent volume enhances the concentration gradient, thereby improving extraction efficiency (Brglez Mojzer et al., 2016). The effect of the solid-to-solvent ratio (w/v) on extraction efficiency is illustrated in Figure 1(b). Experiments were conducted over 240 minutes using 96.5% (v/v) ethanol. Increasing the ratio from 1:20 to 1:30 (w/v) improved extraction efficiency; however, the efficiency declined with further increases in solvent volume. Consequently, a maximum extraction efficiency of 25.6% was achieved at the optimal ratio of 1:30 within the investigated range of 1:20 to 1:50 (w/v). Insufficient solvent volume fails to completely dissolve the target compounds and increases the risk of thermal degradation due to localized heating in the boiling flask. Conversely, an excessive solvent volume prolongs the evaporation-condensation cycle in the Soxhlet apparatus, reducing the frequency of solvent circulation and overall extraction efficiency (Zainurin et al., 2020). Therefore, a solid-to-solvent ratio of 1:30 (w/v) was selected for subsequent studies.

Figure 1. Effects of Soxhlet extraction parameters on extraction yield: (a) Extraction time, (b) solid-to-solvent ratio, (c) Ethanol concentration



Source: Author's calculation

3.1.3. Effect of ethanol concentration on the amount of extract

Figure 1(c) illustrates the extraction yield obtained using ethanol at various concentrations, with the extraction time (240 min) and solid-to-liquid ratio (1:30, w/v) held constant. As the ethanol concentration increased from 50% to 96.5% (v/v), the extraction yield reached a maximum of 35.0% at a concentration of 70% (v/v). Adjusting the ethanol concentration alters the solvent polarity, thereby directly influencing the extraction yield; this variation occurs because the solubility of compounds within the *Ipomoea alba* L. matrix changes according to solvent polarity. Non-polar compounds dissolve readily in non-polar solvents, whereas polar compounds preferentially dissolve in polar media (Jadhav et al., 2009).

3.2. In Vitro insecticidal efficacy of nine insecticide formulations

Nine pesticide formulations were developed based on three main components: Neem leaf extract, *Ipomoea alba* L. extract, and Niaouli oil residue. The total content of these components was 15% (w/v). Specific concentration ranges were set at 6–9% (w/v) for *Ipomoea alba* L. extract and 3–6% (w/v) for neem leaf extract, with the remainder allocated to the oil residue (calculated as 15% minus the combined percentages of the neem leaf and *Ipomoea alba* L. extracts). The active ingredient content used in this study was relatively low compared to similar studies on plant-based pesticide formulations.

- In Vitro insecticidal efficacy of spray-based bioassays

Table 3. Mortality Rate (%) of Nine insecticide formulations

Formulation	Insecticidal Efficacy (%) at Selected Post-Spraying Intervals (min)			Formulation	Insecticidal Efficacy (%) at Selected Post-Spraying Intervals (min)		
	30	120	150		30	120	150
1	10	30	40	6	30	30	40
2	20	20	20	7	0	10	20
3	30	40	40	8	20	30	30
4	60	70	80	9	70	90	100
5	10	30	50				

Source: Author's calculation

As shown in Table 3, Formulation 9 demonstrated the strongest and most rapid insecticidal activity; its composition consisted solely of NE (6%, w/v) and IE (9%, w/v), without any oil residue. After just 30 minutes of exposure, 70% of the mealworms had died; the mortality rate reached 90% after 120 minutes, and 100% mortality was achieved after 150 minutes. Formulation 4 also showed high efficacy; composed of NE (3%, w/v), IE (7.5%, w/v), and oil residue (4.5%, w/v), it achieved an 80% mortality rate within 180 minutes. This formulation contained only half the amount of NE found in Formulation 9, as well as a lower quantity of IE, indicating that the essential oil residue also contributes to insecticidal activity. Furthermore, the essential oil residue possesses antimicrobial properties. Consequently, Formulation 4 was ultimately selected for evaluation. Compared to the previously documented MT1 insecticide formulation containing water spinach, Formulation 4 was identified as the more effective candidate (Lan Oanh et al., 2000).

- In Vitro insecticidal efficacy via leaf-dip bioassay

The mortality rates for the feed-dipping method (Table 4) show similarities to the results obtained with the spraying method (Table 3). Formulation 9 demonstrated the highest efficacy, followed by Formulation 4. Furthermore, clear signs of inhibited feeding activity in the mealworms were observed.

Table 4. Mortality rate (%) of nine insecticide formulations

Formulation	Mortality Rate (%) at Selected Post-Exposure Intervals (h)		Formulation	Mortality Rate (%) at Selected Post-Exposure Intervals (h)	
	24	36		24	36
1	10	10	6	30	30
2	00	20	7	50	60
3	60	70	8	30	30
4	70	80	9	80	100
5	40	40			

Source: Author's calculation

4. Conclusion

Ipomoea alba L. is a vigorous, wild-growing plant with insecticidal properties. Optimal conditions for Soxhlet extraction were determined to be an extraction time of 240 minutes, a solid-to-solvent ratio of 1:30 (w/v), and an ethanol concentration of 70% (v/v), yielding a maximum recovery of 35.0%. A biopesticide formulation comprising neem leaf extract (3%, w/v), *Ipomoea alba* L. extract (7.5%, w/v), and niaouli oil residue (4.5%, w/v) demonstrated effective pest control capabilities. Future research should involve field trials in vegetable-growing areas to validate these results and explore the development of the formulation into commercial biopesticide products in either powder or liquid form ■

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