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Ethnomedicinal and cytotoxicity study of plants used by Dumagat Tribe in Philippines

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Abstract. *Domingo MLM, Gabales M, Guerra DAA, Lindo JSRM, Brillo SC. 2024. Ethnomedicinal and cytotoxicity study of plants used by Dumagat Tribe in Philippines. Asian J Ethnobiol 7: 90-104.* Many indigenous tribes lack access to modern medicine and rely on ethnomedicinal plants to treat various medical conditions. This study documented the ethnomedicinal knowledge and practices of the Dumagats in Barangays San Lorenzo, Norzagaray, and Kabayunan, Doña Remedios Trinidad, Bulacan, Philippines. The study identified 22 ethnomedicinal plants used to treat everyday ailments, with leaves being the most frequently utilized plant part, typically decocted and taken orally. Eighteen out of 22 (68.18%) of the plants were identified to be native to the Philippines: *Alstonia scholaris, Homalomena philippinensis, Blumea balsamifera, Combretum indicum, Dillenia philippinensis, Pterocarpus indicus, Flagellaria indica, Cyrtandra incisa, Leea philippinensis, Lagerstroemia speciosa, Saccharum spontaneum, Embelia philippinensis, Antidesma bunius,* and *Buddleja asiatica* highlight the predominance of native plants in the area and its utilization by the tribe. The most frequently used plants were *Artemisia vulgaris, B. balsamifera, S. spontaneum,* and *D. philippinensis*, with a use value 0.57. The results shed light on the pharmacological characteristics of plant extracts from *A. vulgaris, C. amboinicus,* and *B. balsamifera* using brine shrimp lethality and trypan blue assays. Examination of concentration and time-dependent effects revealed cytotoxic properties. Higher concentrations (1 mg/mL) significantly reduced cell viability, while lower concentrations (100 and 10 micrograms/mL) showed varied responses; prolonged exposure exacerbated cytotoxic effects. These findings underscored the importance of documenting ethnomedicinal practices and assessing the cytotoxicity of medicinal plants to understand their potential health impacts, providing practical insights for researchers, botanists, and healthcare professionals interested in ethnomedicine and pharmacology.

Keywords: Assay, cytotoxicity, Dumagats, ethnomedicinal, plants

Abbreviations: BSLA: Brine Shrimp Lethality Assay, LC_{50}: Lethal Concentration 50, TBA: Trypan Blue Assay

INTRODUCTION

Plants serve as the primary source for discovering new pharmacologically active substances in the pharmaceutical industry, leading to the development of many medicines. This benefits both conventional medicine and the global healthcare system by providing raw materials for drug development, supporting traditional medicine, and contributing to biodiversity and ecosystem stability. Additionally, plants offer sustainable alternatives to synthetic drugs and enhance healthcare access, especially in developing regions, improving global health outcomes. Roberson (2008) stated that between 50,000 and 80,000 flowering plants are used for medicinal purposes worldwide. The Philippines boasts diverse plant species and numerous ethnic groups with distinct cultural traditions (Dapar et al. 2020). Given the Philippines' wide range of medicinal plant species, researchers and medical experts can offer the nation and its people more affordable and accessible healthcare, particularly in impoverished and remote areas without modern medical facilities.

De Vera (2007) conducted a country case study on the Philippines, revealing the presence of 110 significant indigenous tribes and 112 ethnolinguistic groups. The indigenous people continue to rely on upland areas for their traditional agricultural practices. Over generations, traditional cultures effectively shared knowledge about using plants for curing ailments. Despite the archipelago's various indigenous tribal populations and their traditional medicine practices, there is a lack of comprehensive research on ethnomedicine, particularly regarding the cytotoxicity of plants used in the Philippines. Ethnomedicine focuses on cultural interpretations of health, disease, and illness and aims to address the healthcare process and healing practices (Krippner and Staples 2003; Mahapatra et al. 2019). The deeper integration of younger generations into society is leading to a gradual loss of traditional knowledge. Preserving ethnomedical practices and knowledge in the Philippines faces challenges due to transmitting knowledge through oral traditions, which may require more extensive documentation.

Adriano (2020) described the Dumagats as an indigenous group living in Barangays San Lorenzo, Norzagaray, and Kabayunan, Doña Remedios Trinidad, Bulacan, Philippines near the Angat watershed and the Sierra Mountains. A dedicated missionary, Martin Francisco played a critical role in the Dumagats' establishment. The Kabuwelan phrase "*hubad sa gubat*"

(naked in the forest) is the source of the word "*dumagat*," which refers to the small community where the Dumagats reside. Adriano (2020) also mentioned the Agta, Alta, and Remontado Tribes, three of the Dumagat Tribes. Access to clean water is a pressing concern for the Dumagats, leading to amoeba-borne diseases and other health problems.

The risks faced by ethnomedicinal knowledge and practices in the Philippines motivated the researchers to conduct a cytotoxicity assay and document ethnomedicinal plants. These risks include the lack of information on traditional medicine, which can lead to indigenous people being unaware of the potentially harmful and fatal cytotoxic activity of certain plants. Furthermore, there is limited information available on the Dumagats and their medical practices, prompting researchers to conduct a study with the Dumagat community in Barangays San Lorenzo, Norzagaray, and Kabayunan, Doña Remedios Trinidad, Bulacan.

The study aimed to explore the cytotoxicity of specific ethnomedicinal plants in Norzagaray and Doña Remedios Trinidad, Bulacan. It utilized local ethnomedical knowledge and practices in the barangays of San Lorenzo and Kabayunan. The study also focused on the importance and diversity of traditional medical applications by identifying and documenting the locally used medicinal plants. The study's findings, with their potential to inspire and guide future research, provide valuable insights for future research.

MATERIALS AND METHODS

Research design

This study employed non-experimental and experimental quantitative research methods, focusing on practical applications. Survey research, a common tool in health services research, describes Dumagats' practices, including the plant parts they use, their preparation and application methods, and the ailments they claim to cure. Experimental methods, such as cytotoxicity assays like the BSLA and TBA, further enhance the practicality of the study.

Research locale

The Dumagat Tribe of Barangays San Lorenzo, Norzagaray, and Kabayunan, Doña Remedios Trinidad, Bulacan, Philippines participated in this study (Figure 1). Kabayunan is located by maps at approximately 14.9440, 121.2688, in the municipality of Doña Remedios Trinidad, while San Lorenzo is located at approximately 14.8664, 121.2398, in the municipality of Norzagaray (Figure 2). Due to the presence of Dumagats in these barangays in Norzagaray and Doña Remedios Trinidad, Bulacan, it was chosen as the study's site.

Samples and sampling procedures

We conducted this study from January 2023 to June 2024. Researchers used nonprobability and purposive sampling. Considering the number of participants and the community, the researchers have determined that purposive sampling is the most appropriate method for the study. According to Allen (1971), one way to determine how to select informants is to establish criteria for identifying a trustworthy informant. We construct a list of qualifications based on these criteria. The researchers provided the list of qualifications to resource people (community leaders, local government officials, or residents) who can assist in finding informants. This research aims to highlight the benefits of the research to the community, thereby reducing time and preventing conflicts during data collection (Allen 1971; Bernard et al. 1986). We asked individual resource persons to identify the eight most pertinent informants. The goal is to identify the most highly referenced individual who will serve as an informant (Sanders 1960). We have adopted the purposeful sampling method because it best suits ethnomedicinal research, which involves studying cultural traits unknown to all participants.

Figure 1. The general community of Dumagat Tribes in Norzagaray and Doña Remedios of Bulacan, Philippines

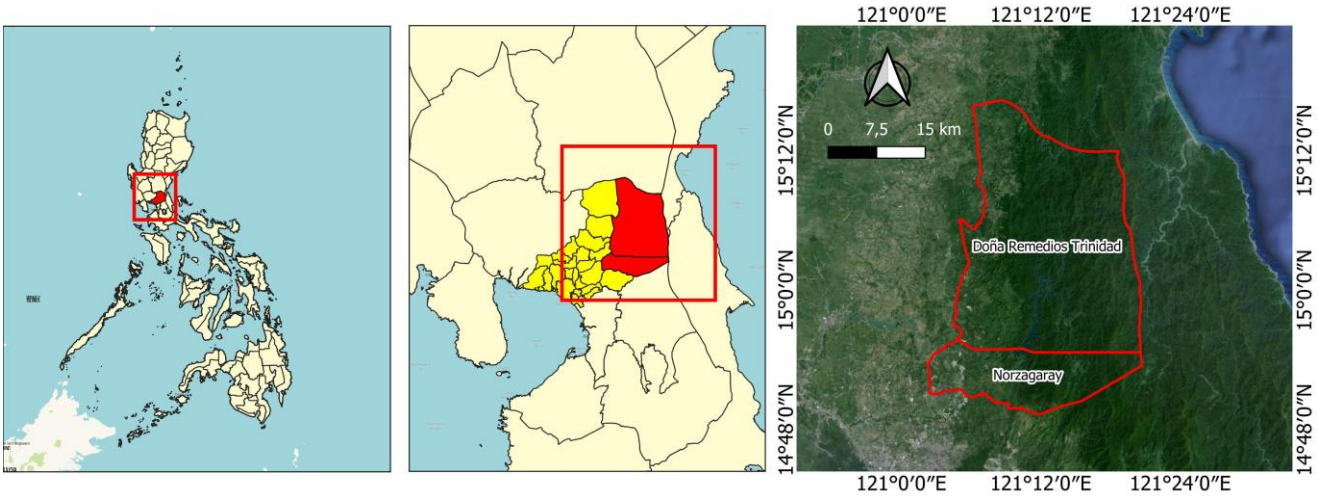

Figure 2. Study area in Norzagaray and Doña Remedios of Bulacan, Philippines

Inclusion and exclusion criteria

The informants in this study are 18-80 years old, male and female, and part of the Dumagat Tribe. These individuals are native and have at least 5 years of experience using or applying plants to cure ailments to themselves or others in the study area, resulting in a favorable reputation. Regarding barangays, only the two—Barangays San Lorenzo, Norzagaray, and Kabayunan—and Doña Remedios Trinidad in Bulacan were selected to be part of the study due to the interest and accessibility of researchers to the population. Plant inclusion criteria are based on selecting plants within the research locale that are either spontaneous or cultivated; the informants mention any plant parts they use to treat ailments. We exclude informants who are non-Dumagats, have less than 5 years of experience, or are non-herbalists. We also exclude Norzagaray's remaining barangays from the study.

Methods of collecting data and research instrumentation

Before conducting the study, the local government of Norzagaray, Bulacan, received an informed consent form. We then conducted semi-structured interviews and focus group discussions to gather ethnomedicinal knowledge. A semi-structured questionnaire guided the interview with the informants. In addition, we conducted the semi-structured interview in Tagalog, given that the Dumagats have acquired the ability to speak and communicate in this language due to the internal migration of other ethnolinguistic groups into their territory.

Plant collection

We collected the plant specimens mentioned by the informants. The researchers further identified the plant specimens using various book references, including A Pictorial Cyclopedia of Philippine Ornamental Plants by Madulid (1995), Co's Digital Flora of the Philippines (updated 2024, Pelser et al. 2011), and Stuart's (2024) List of Philippine Herbal and Medicinal Plants. Plant specimens were identified and verified at the University of the Philippines—Diliman.

Plant preservation

The plant specimens were preserved using Queensland Herbarium's (2013) Collection and Preservation of Plant Specimens. We washed the specimens with denatured alcohol to prevent molding. We individually placed the specimens between layers of newspapers and pressed corrugated boards between wooden pressers. We preserved the plants using a plywood press and checked their daily status. Once the plant had thoroughly dried, we mounted it on a bristol board and covered it with a plastic cover. We sent the herbarium to the Jose Vera Santos Memorial Herbarium at the University of the Philippines—Diliman for storage and future reference for identification.

Plant extraction

A kilogram of each fresh plant part from the three selected species was collected to prepare crude extracts for the brine shrimp lethality assay. The fresh plant parts were washed with tap water to remove unwanted particles and oven-dried at 44.5°C for 4 hours. The dried plant samples were cut into small pieces and pulverized using a blender (600 W, model NB-101B, Nutribullet 600 series). The obtained powder from each plant species was weighed using an analytical balance. The maceration procedure from Ang et al. (2019) was slightly modified by soaking powdered dry plant leaf samples in sufficient amounts of 95% ethanol for 24 hours at ambient room temperature. The samples were then filtered twice using Whatman filter paper, and the collected filtrates were rotary-evaporated at 40°C to remove the solvent (ethanol). Before experimental use, the dried crude ethanol extracts were kept at 4°C (Selvamohan et al. 2012). The serial dilution procedure from Sarah et al. (2017) was also slightly modified. A stock solution was prepared by dissolving 10 mg of the powder in 1 mL of water, followed by serial dilution to obtain concentrations of 1 mg/mL, 100 µg/mL, and 10 µg/mL.

Clean, properly labeled test tubes were used for this preparation.

Brine Shrimp Lethality Assay

The brine shrimp lethality assay, adapted from Sarah et al. (2017), assessed the cytotoxicity of plant extracts. Researchers prepared artificial seawater by mixing 27 g of table salt with 3 L of water in a rectangular tank and aerated it with an air pump. Then, 15 g of brine shrimp eggs were added and incubated under a light source (60-100-watt bulb) for 20-24 hours to hatch nauplii. After another 24 hours, the nauplii were separated from the empty eggs by turning off the air pump and light. Ten nauplii were transferred to test tubes using a Pasteur pipette and exposed to varying concentrations of plant extracts. The number of survivors was recorded every four hours for 24 hours. Positive and negative controls, using potassium dichromate and no extracts, respectively, ensured result accuracy.

Trypan Blue Assay

A yeast broth was prepared by dissolving 11.5 grams of S-04 *Saccharomyces cerevisiae* in 240 mL of warm water, and 1 mL of this slurry was diluted with 10 mL of distilled water. Following Kamiloglu et al. (2020), 1 mL of plant extracts at concentrations of 1 mg/mL, 100 µg/mL, and 10 µg/mL was mixed with 1 mL of diluted yeast slurry. The mixture was observed at 15, 30, and 45 minutes, then combined with 2 mL of 0.4% trypan blue stain and left at room temperature for 3 minutes. After incubation, a mixture drop was placed on a microscope slide to count viable (unstained) and nonviable (stained) cells.

Statistical analysis

The researchers used Use Value (UV) to estimate the relative significance of the plant species used by the Dumagats. According to Zenderland et al. (2019), use value is a commonly employed indicator for ranking the comparative value of plant species. We widely use it to identify notable species of interest because it incorporates the frequency of a particular species' mention and the number of uses described per species.

$$UV = \frac{Ui}{N}$$

Where:

UV: Use Value

Ui: The number of plant usage reports in the research region

N: The overall number of respondents

The Brine Shrimp Lethality Assay evaluated the lethality of plant extracts against *Artemia salina*. The following equation was used to determine the percentage of mortality:

$$\%mortality = \frac{Number\ of\ dead\ nauplii}{Number\ of\ live\ nauplii\ taken} \times 100$$

Data were analyzed using one-way ANOVA in SPSS to identify significant differences in mortality rates at various extract concentrations after 24 hours, with a p-value below 0.05 considered significant. The LC_{50}, the concentration at which 50% of brine shrimp died, was determined using 24-hour mortality data and probit analysis. LC_{50} is a reliable measure, less affected by extreme values, reflecting the median lethal dose. Extracts with LC_{50} values less than 1 mg/mL were considered toxic, while those above 1 mg/mL were non-toxic. Toxicity levels were classified using Clarkson's et al. (2004) criteria: non-toxic (>1.00 mg/mL), mildly hazardous (0.50-1.00 mg/mL), moderately toxic (0.10-0.50 mg/mL), and very toxic (<0.10 mg/mL).

For the trypan blue test, the proportion of live cells (percent viable cells) was computed with the following formula provided by Strober (1997):

$$viable\ cell\ (\%) = \frac{Total\ number\ of\ viable\ cells\ per\ ml\ of\ alliquot}{Total\ number\ of\ cells\ per\ ml\ of\ alliquot} \times 100$$

The percent viability computed for the triplicates at different concentrations under three-time exposures (15, 30, and 45 minutes) was analyzed using a one-way ANOVA to determine if there was a significant difference in the concentration values for each plant extract.

RESULTS AND DISCUSSION

Plants used by the Dumagat Tribe and modes of preparation

Following the interviews made with the informants, presented in Table 1, the 22 medicinal plants belonging to 21 taxonomic families were documented in Sitio Manalo, Barangay San Lorenzo, Norzagaray, Bulacan, and Sitio Iyak, Barangay Kabayunan, Doña Remedios Trinidad, Bulacan. Figure 3 shows that the Asteraceae family represents these plants with two plants, while the other families each record one plant. Dumagats treat 34 ailments with ethnomedicinal plants, according to the study. Colds, coughs, fever, UTI, pneumonia, and mouth sores were the most common diseases treated using medicinal plants in the two Barangays. Cough was the most common ailment documented, with 11 plants, followed by fever with five plants, mouth sores, colds, pneumonia, and UTI with two plants, and the rest of the ailments with one plant. Table 1 shows that Dumagats use specific plants to treat three or more ailments. The Dumagats used some of these plants, such as *Artemisia vulgaris*, commonly known as mugwort and known locally as *damong maria*, to treat cough, hyperacidity, and loss of appetite. *Blumea balsamifera* (*sambong*) also served as a remedy for folk illnesses like numbness, stomach pain, or "*pasma*." *Pasma* is a Filipino folk illness believed to be caused by the sudden exposure of hot or sweaty body parts to cold, leading to symptoms like tremors, muscle spasms, or numbness (Jocano 1973). Meanwhile, *Dillenia philippinensis* (*katmon*) fruit was a remedy for flu-like symptoms. *Alstonia scholaris* (*dita*) fruit was specifically used as a malaria treatment. *Combretum indicum* (*taryantan*) can treat ailments like

coughs, colds, pneumonia, and sprains. This was the most effective medicinal plant, treating four different ailments. The sap of this plant can treat other ailments, such as mouth sores in children and adults, while *talahib*, *lagundi*, *tawa-tawa*, or *tanaw-dagat* can treat coughs and fevers (Lam et al. 2018).

According to Canceran et al. (2021), the leaves of *A. vulgaris* (*damong maria*) were used to treat stomach pain, one of the causes of hyperacidity. Researchers in Bangalore, India, have scientifically studied this traditional remedy, demonstrating its potential to reduce hyperacidity (Zubair et al. 2020). In albino rats, the same pharmacological study revealed the anti-inflammatory effects of flavonoids in *A. vulgaris* leaves. In Aurora, Quezon, people also use *B. balsamifera* (*sambong*) to treat *pasma* (a local term referring to musculoskeletal spasms) and body pain by consuming its leaves orally (Canceran et al. 2021). People apply the plant, which contains compounds with anti-inflammatory and antioxidant properties, directly through heat to relieve muscle pain or strain (Kantasrila et al. 2020). Additionally, Boy et al. (2019) noted that *B. balsamifera* is among the medicinal plants approved by the Department of Health, Cubans with a persistent cough or tuberculosis received a decoction from the leaves. Researchers discovered that *C. amboinicus* (*oregano*) effectively combats *Mycobacterium tuberculosis* (Arumugam et al. 2016).

Regarding ethnomedicinal knowledge, it's important to note that all informants have used medicinal plants for over five years and inherited knowledge of their preparation and application from their parents, siblings, and ancestors. This inheritance is a sign of the informants' deep respect for their ancestors and cultural heritage, a connection that the audience can surely relate to. The informants mainly found the herbal plants they used in the surrounding environment. In addition, plants are widely available throughout the year and are easy to reach. Seasonal variations usually do not affect the plants mentioned by informants, indicating their availability throughout the year (Balinado and Chan 2017). Apart from the ethnomedicinal knowledge mentioned previously, informants also documented the use of herbal plants, the diseases they believe can be treated, how they are made, and how they are applied. Table 1 summarizes data regarding these traditional healthcare practices.

The Dumagats utilized various plant parts, such as the stem, trunk, sap, roots, fruit, heart of palm, bark, and leaves. In Figures 5, 6, and 7, plant species can fall into one or more categories if such plant part is used in such application or category. As shown in Figure 4, leaves are the most used plant part by Dumagats, accounting for 41% of the ethnomedicinal practices. This is also the case with the Dumagats of Casiguran, Aurora, where leaves are the most utilized plant for ethnomedicinal practices (Canceran et al. 2021). Bark accounted for 17% of Dumagats' ethnomedicinal practices, followed by fruit at 10%. The palm's trunk, roots, sap, and heart came next with 7% each, while the stem was the least used plant part, with 4% out of all the parts. According to the study by Gnanaraj et al. (2016), the leaves of *Flagellaria indica* (*baling-uai*) are believed by some to have additional medicinal benefits, including treating cough and vomiting. Furthermore, people consume the roots of *F. indica* to treat vomiting, influenza, and coughs (Haris et al. 2022).

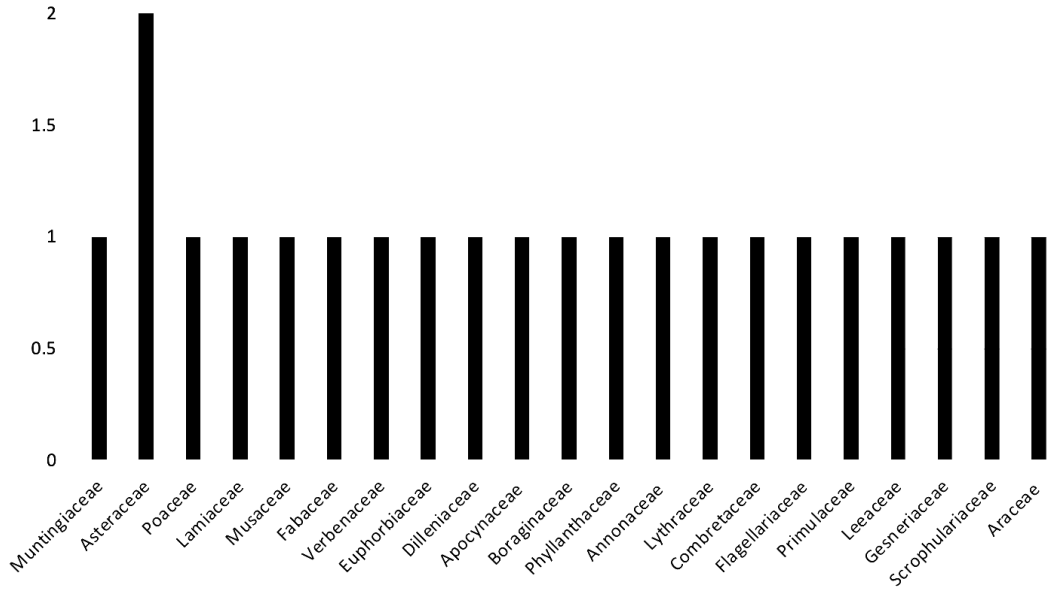

Figure 3. Family of ethnomedicinal plants used by Dumagats of two selected barangays in Philippines

Table 1. List of ethnomedicinal plants used by Dumagats of two selected barangays in Philippines

Family[1]	Scientific name[1]	Local name	Common name	Plant part used[2]	Mode of preparation[2]	Mode of application[2]	Ailment[2]	Conservation Status[3, 4]	Distribution record[3]
Annonaceae	*Annona muricata* L.	*Guyabano*	Soursop	Leaves	Decoction	Taken orally	Diabetes, pneumonia	Not Evaluated	Central America, Caribbean
Apocynaceae	*Alstonia scholaris* (L.) R.Br.	*Dita*	Blackboard tree	Fruit	Decoction	Taken orally	Malaria	Not Evaluated	Australia, Borneo, China, India, Laos, Luzon, Mindanao, Visayas
Araceae	*Homalomena philippinensis* Engl.	*Tagupos*	Payau, emerald gem	Leaves	Directly heated	Taken orally	Cough	Not Evaluated	Luzon, Mindanao, Taiwan
Asteraceae	*Artemisia vulgaris* L.	*Damong maria*	Mugwort	Leaves	Directly heated	Taken orally	Cough, hyperacidity, Loss of appetite	Not Evaluated	Negros, Panay (Pantropic)
	Blumea balsamifera (L.) DC.	*Sambong*	Ngai camphor, sembung	Leaves	Directly heated	Direct application	Folk illness, numbness, stomach pain	Least Concern	Australia, Borneo, China, India, Java, Luzon, Mindanao, Visayas
Boraginaceae	*Cordia dichotoma* G. Forst.	*Anonang*	Indian cherry	Bark	Decoction	Taken orally	Fever, relapse	Not Evaluated	Borneo, Java, Luzon, Mindanao, New Guinea, Singapore, Sumatra, Visayas
Combretaceae	*Combretum indicum* (L.) DeFilipps	*Taryantan*	Rangoon creeper	Bark	Decoction	Taken orally	Cough, colds, Pneumonia, sprain	Not Evaluated	Bangladesh, China, India, Laos, Luzon, Mindanao, Myanmar, Visayas
Dilleniaceae	*Dillenia philippinensis* Rolfe	*Katmon*	Philippine dillenia, philippine katmon, elephant apple	Fruit	Directly heated, Decoction	Direct application	Cough, colds, fever	Not Evaluated	Luzon, Mindanao, Visayas
Euphorbiaceae	*Euphorbia hirta L.*	*Tawa-tawa*	Asthma weed, asthma plant, hairy spurge	Roots	Decoction	Taken orally	Cough, fever	Not Evaluated	America, Luzon, Visayas
Fabaceae	*Pterocarpus indicus* Willd.	*Narra*	Angsana	Trunk, Sap	Sap extraction	Direct application	Mouth sore	Vulnerable	Cambodia, Luzon, Malesia, Mindanao, Myanmar, Thailand, Visayas
Flagellariaceae	*Flagellaria indica* L.	*Baling-uai*	Whip vine	Heart of palm	Directly heated	Taken orally	Cough	Not Evaluated	Africa, Borneo, Cambodia, China, India, Luzon, Mindanao, Visayas
Gesneriaceae	*Cyrtandra incisa* C.B. Clarke in DC	*Katampas*	Katampas	Roots, Leaves	Decoction	Direct application	Indigestion	Not Evaluated	Luzon
Lamiaceae	*Coleus amboinicus* Lour.	*Oregano*	Indian borage, cuban oregano, mexican mint, spanish thyme	Leaves	Steamed, pounding	Direct application	Cough	Not Evaluated	Luzon

Leeaceae	*Leea philippinensis* Merr.	*Makasdo*	West indian holly	Leaves	Directly heated	Taken orally	Cough	Not Evaluated	Luzon, Mindanao, Taiwan, Visayas
Lythraceae	*Lagerstroemia speciosa* (L.) Pers.	*Banaba*	Queen crepe myrtle, rose of india	Fruit, leaves, bark	Decoction	Taken orally	Urinary tract infection	Not Evaluated	Borneo, Cambodia, China, India, Laos, Luzon, Mindanao, Visayas
Muntingiaceae	*Muntingia calabura* L.	*Aratiles*	Jamaica cherry	Bark	Decoction	Taken orally	Dysentery	Not Evaluated	Luzon, Mindanao, Visayas
Musaceae	*Musa* sp.	*Saging matsing*	Banana	trunk	Directly heated	Direct application	Mouth sore	Not Evaluated	Borneo, China, India, Java, Laos, Luzon, Mindanao, Visayas
Poaceae	*Saccharum spontaneum* L.	*Talahib*	Wild sugarcane	Leaves, stem	Directly heated, pounding	Taken orally, direct application	Cough, fever	Not Evaluated	Africa, Australia, Luzon, MIndanao, Visayas
Primulaceae	*Embelia philippinensis* A. DC.	*Lando*	Dikai	Leaves	Directly heated	Direct application	Fever	Not Evaluated	Luzon, Mindanao, Visayas
Phyllanthaceae	*Antidesma bunius* (L.)	*Bignay*	Chinese laurel	Bark	Decoction	Taken orally	Urinary tract infection	Not Evaluated	Borneo, China, India, Java, Laos, Luzon, Mindanao, Visayas
Scrophulariaceae	*Buddleja asiatica* Lour.	*Tanaw dagat*	Dog tail, malasambung, butterfly bush	Leaves	Directly heated	Taken orally	Cough, fever	Not Evaluated	China, India, Luzon, Malesia, Mindanao, Pakistan, Taiwan, Visayas
Verbenaceae	*Vitex negundo* L.	*Lagundi*	Chaste tree	Leaves	Decoction	Taken orally	Cough, fever	Not Evaluated	China, India, Java, Luzon, Mindanao,

Note: [1]Jose Vera Santos Memorial Herbarium verified data at the University of the Philippines, Diliman, [2]Data were derived from the ethnobotanical survey of informants, [3](Pelser et al. 2011), [4](IUCN 2024)

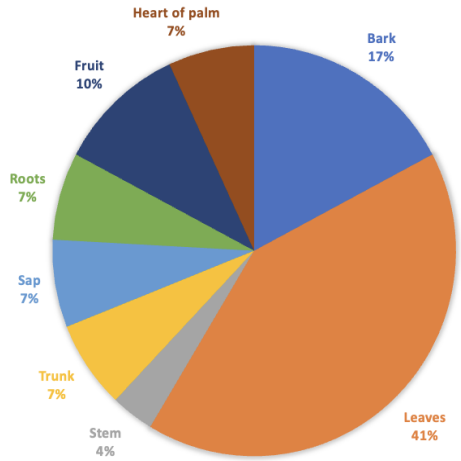

Figure 4. Percentage analysis of plant parts used for ailments treated

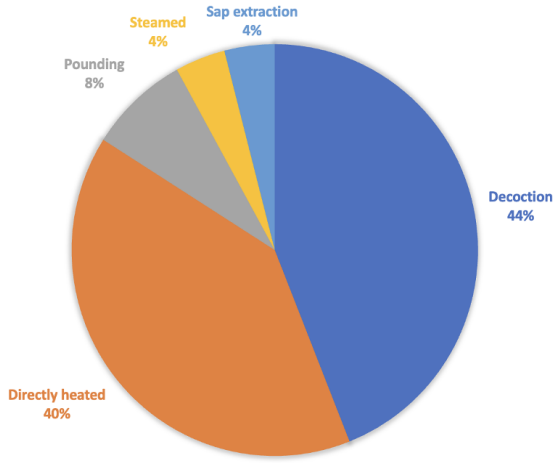

Figure 5. Percentage analysis of methods of preparation by Dumagats in Philippines

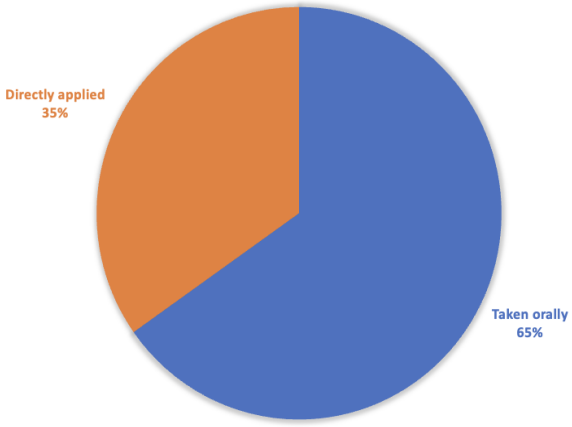

Figure 6. Percentage analysis of methods of application by Dumagats in Philippines

In Figure 5, the preferred preparation method for Dumagats is decoction (44%), followed by direct heating (40%). This is consistent with other Dumagats in Casiguran, Aurora (Canceran et al. 2021). Ethnomedicinal plants in Sitio Manalo and Sitio Iyak are commonly boiled. *V. negundo* (*lagundi*) and *L. speciosa* (*banaba*) are boiled for 30 minutes. The hearts of *F. indica* (*baling-uai*) and *S. spontaneum* (*talahib*) are heated until sap appears. *D. philippinensis* (*katmon*) fruit is either decocted or directly heated. Pounding is used to obtain plant extracts, notably for *S. spontaneum* and *C. amboinicus* (*oregano*). Steaming and cutting are the least used methods. *P. indicus* (*narra*) is prepared by cutting the trunk for sap. *C. amboinicus* is also steamed over rice. The Dumagats obtain a sap extract of *Pterocarpus indicus* (*narra*) to treat mouth sores (Canceran et al. 2021). Furthermore, Dharmaraj et al. (2022) views *P. indicus* as a potential source of natural anti-inflammatory substances that could aid in treating inflammation.

Administration methods can be internal or external; of 22 reported medicinal plant species, 65% are taken orally, whereas the remaining 35% are applied directly to the skin, as seen in Figure 6. The most preferred way of administering the plant is through an oral route. Since cough is the most common ailment, people typically consume the plants orally. Hussaqin et al. (2014) and Hassan et al. (2024) afound that *Saccharum spontaneum* (*talahib*) leaves have the potential to treat respiratory problems, and the stems have demonstrated efficacy in addressing general debility. *Annona muricata* (*guyabano*) is one of the medicinal plants studied in Tarlac that has an antihyperglycemic effect (Mina and Mina 2017). The same study used the leaves of *A. muricata* for a tea decoction. Different plant parts of *Muntingia calabura* (*aratiles*) are used as antiseptics, diuretics, and laxatives. Chaudhari et al. (2020) also uses this to treat conditions like diarrhea, dysentery, and asthma.

The use of medicinal plants is a testament to the individualized nature of healthcare. These plants are usually applied directly to affected body areas, and there are no standard measurements for administering herbal remedies. Instead, dosages are tailored to each patient, considering their age, condition, and level of relief. For instance, young people may receive less than adults. This personalized approach respects the unique needs of each patient. *Musa* sp. is a prime example of this, as it can potentially treat anti-inflammatory ailments using several plant parts, including the leaf, sap, and trunk (Yadav 2021).

Based on the consolidated data in Table 2, the following plants had the highest UV in the two barangays: *A. vulgaris* (UV = 0.57), *S. spontaneum* (0.57), *B. balsamifera* (0.57), and *D. philippinensis* (0.57). This demonstrates these plants' extensive use, high value, and perceived effectiveness in the reported barangays. Plant species sensitive to high UV levels face intense harvest pressure and require extra conservation measures. Most medicinal plants used in the research area are confirmed to be cultivated, ensuring they do not threaten to pose a threat to their wild counterparts (Albuquerque et al. 2006).

Table 2. Number of Use Reports (UR) and its calculated Use Value (UV) of reported ethnomedicinal plants

Family	Scientific name	Local name	Use reports[1]	Use value[1]
Asteraceae	*Artemisia vulgaris* L.	*Damong maria*	4	0.57
	Blumea balsamifera (L.) DC.	*Sambong*		
Dilleniaceae	*Dillenia philippinensis* Rolfe	*Katmon*		
Poaceae	*Saccharum spontaneum* L.	*Talahib*		
Fabaceae	*Pterocarpus indicus* Willd.	*Narra*	2	0.28
Flagellariaceae	*Flagellaria indica* L.	*Baling-uai*		
Lamiaceae	*Coleus amboinicus* Lour.	*Oregano*		
Muntingiaceae	*Muntingia calabura* L.	*Aratiles*		
Musaceae	*Musa* sp.	*Saging matsing*		
Verbenaceae	*Vitex negundo* L.	*Lagundi*		
Annonaceae	*Annona muricata* L.	*Guyabano*	1	0.14
Apocynaceae	*Alstonia scholaris* (L.) R.Br.	*Dita*		
Araceae	*Homalomena philippinensis* Engl.	*Tagupos*		
Boraginaceae	*Cordia dichotoma* G. Forst.	*Anonang*		
Combretaceae	*Combretum indicum* (L.) DeFilipps	*Taryantan*		
Euphorbiaceae	*Euphorbia hirta L.*	*Tawa-tawa*		
Gesneriaceae	*Cyrtandra incisa* C.B. Clarke in DC	*Katampas*		
Leeaceae	*Leea philippinensis* Merr.	*Makasdo*		
Lythraceae	*Lagerstroemia speciosa* (L.) Pers.	*Banaba*		
Phyllanthaceae	*Antidesma bunius* (L.)	*Bignay*		
Primulaceae	*Embelia philippinensis* A. DC.	*Lando*		
Scrophulariaceae	*Buddleja asiatica* Lour.	*Tanaw dagat*		

Note: [1](Zenderland et al. 2019)

Table 3. LC_{50} was obtained using Brine Shrimp Lethality Assay in 6, 12, 18, and 24 hours post-treatment of the plant extracts and controls

Treatment	Time			
	6 hours	12 hours	18 hours	24 hours
Potassium dichromate	0.000	0.000	0.000	0.000
A. vulgaris	0.953	0.859	0.732	0.706
C. amboinicus	0.832	0.685	0.591	0.338
B. balsamifera	1.000	1.000	0.826	0.706
No concentration	1.000	1.000	0.900	0.800

Brine Shrimp Lethality Assay

The present study assesses the cytotoxicity of selected ethnomedicinal plants used by the Dumagats. The results reveal cytotoxicity in plant extracts and other compounds based on their ability to cause death in lab-cultured brine shrimp larvae (nauplii), supporting the use of chosen ethnomedicinal plants in traditional medicine.

Table 3 displays the LC_{50} values obtained through experimentation with brine shrimp as test subjects. The results of the current study were comparable to Waghulde et al. (2019), who found that the extent of lethality is directly proportional to the extract's concentration. The constant LC_{50} values of 1 mg/mL at 6 hours and 12 hours, 0.900 mg/mL at 18 hours, and 0.800 mg/mL at 24 hours indicate that the brine shrimp in the control group (no concentration) did not experience any adverse effects within the given time frames. Among the substances tested, potassium dichromate demonstrates a remarkably high level of toxicity, as evidenced by an LC_{50} value of 0.00000 mg/mL at 6 hours of exposure. Additionally, the data indicates a time-dependent increase in toxicity for *A. vulgaris*, as indicated by the decreasing LC_{50} values over longer exposure periods. Specifically, a concentration of 0.953 mg/mL was lethal to 50% of the brine shrimp within 6 hours, while concentrations of 0.859 mg/mL, 0.732 mg/mL, and 0.706 mg/mL caused similar mortality rates within 12, 18, and 24 hours, respectively.

Inducing *C. amboinicus* resulted in an LC_{50} value of 0.832 mg/mL at 6 hours, indicating that about half of the brine shrimp population died at that concentration. At 12 hours, the value dropped to 0.685 mg/mL, suggesting a lower concentration was required to maintain the same mortality rate. The concentration dropped to 0.591 mg/mL after 18 hours and 0.338 mg/mL after 24 hours. The results for *B. balsamifera* showed that it was toxic to brine shrimp for about 6 to 12 hours, with an LC_{50} value of 1 mg/mL at both times. This means that the same concentration was needed to get a 50% death rate. However, at 18 hours, the LC_{50} value decreased to 0.826 mg/mL, indicating that a lower concentration of *B. balsamifera* was required to achieve the same effect. Furthermore, at 24 hours, the LC_{50} value decreased further to 0.706 mg/mL, suggesting that the brine shrimp's sensitivity to *B. balsamifera* exposure increased over time.

Findings from the brine shrimp lethality assay show that the survival rates of the negative control group (no concentration) stayed at the expected level. This is because there was no substance or experimental treatment in that group. This provides a baseline for comparison when evaluating the potential toxicity or effects of substances tested in the experimental group. On the other hand, results for the positive control suggest that even minute concentrations of potassium dichromate can lead to a considerable reduction in the brine shrimp population within a specified timeframe.

Findings also suggest prolonged exposure to *A. vulgaris* results in a higher toxicity level for the brine shrimp population. As for *C. amboinicus* and *B. balsamifera*, the LC_{50} values demonstrated that the brine shrimp's sensitivity to both plant extract exposures increased as the duration of exposure increased. Waghulde et al. (2019) also found that the shrimp started to die after prolonged exposure to higher concentrations of treatment extracts.

We used Clarkson's et al. (2004) criteria to infer the toxicity of extracts tested in the brine shrimp lethality assay. Clarkson et al. (2004) categorized the extract as non-toxic at concentrations greater than 1.00 mg/mL, mildly hazardous around 0.50 and 1.00 mg/mL, moderately toxic within 0.10 and 0.50 mg/mL, or very toxic below 0.10 mg/mL. Therefore, based on the results after 24 hours, potassium dichromate is considered very toxic, *A. vulgaris* is mildly hazardous, *C. amboinicus* is moderately toxic, and *B. balsamifera* is also mildly hazardous. As expected, no concentration is non-toxic.

***Artemisia vulgaris* (*damong maria*)**

As stated in the study by Chan and Lin (2010), *A. vulgaris* contains thujones, which can be the reason for its toxicity. Aronson (2016) found in another study that *A. vulgaris* contains the toxic lactone santonin, once used as an anthelmintic drug to kill or stun parasitic worms but now replaced by less toxic compounds. According to Scott (2005), lactones can act as sedatives and antispasmodics; however, some lactones exhibit neurotoxic effects and can also cause skin sensitizing or irritation. On the other hand, santonin is a sesquiterpene lactone most frequently found in the Asteraceae plant and isolated from santonin-containing *Artemisia* species (Wedge et al. 2000). Based on the same study by Aronson (2016), depending on the origin of the plant, major components can be 1,8-cineole, camphor, linalool, and thujone.

According to Hoch et al. (2023), 1,8-cineole (Eucalyptol) clinical applications include treating rhinosinusitis, chronic obstructive pulmonary disease, asthma, and bronchitis. Meanwhile, medicinal practitioners use camphor as an antipruritic, mild analgesic, and counterirritant. However, camphor can cause generalized seizures because it acts as a CNS stimulant. It also acts as a local mucosal irritant and can cause hepatic failure in severe intoxications (Adkins 2024). In the psychopharmacological evaluation of mice, Elisabetsky (2002) revealed anticonvulsant properties (dose-dependent, marked sedative effects at the CNS). Romm et al. (2010) found that internal consumption of thujone, as practiced by the Dumagats, can be neurotoxic, convulsant, and hallucinogenic. Long-term and excessive use of thujone-rich products can cause restlessness, vomiting, vertigo, tremors, renal damage, and convulsions.

In 2016, Judzentiene and Garjonyte (2016) investigated the different parts of *A. vulgaris* essential oils and their toxicity. They found that the main parts were davanones (13.8-45.5%, six oils), germacrene D (9.1-30.5%, four oils), 1,8-cineole (16.4%, one oil), camphor (18.9%, one oil), trans-thujone (8.9 and 10.9%, two oils), and cis-chrysanthenyl acetate (10.4%, one oil). Judzentiene and Garjonyte (2016) were the first to describe *A. vulgaris* davanone chemotype and obtain LC_{50} values using the brine shrimp assay. The results, after 24 hours of exposure, revealed that the oils containing appreciable amounts of germacrene D, 1,8-cineole, camphor, and davanone were notably toxic, of which two of the components (1,8-cineole and camphor) were also previously mentioned.

***Blumea balsamifera* (*sambong*)**

Masyudi et al. (2022) used ethanol and ethyl acetate to test sambong extracts for phytochemicals. They found that steroids, flavonoids, phenolics, and phenolics were present. Pang et al. (2014) have isolated 100 volatile and non-volatile constituents from *B. balsamifera*. Its volatile constituents, which were the primary active ingredients, contain most of these components. These volatile constituents encompass a range of compounds, including terpenoids, fatty acids, phenols, alcohols, aldehydes, ethers, ketones, pyridines, furans, and alkanes. On the other hand, flavonoids, such as flavonoid, flavanone, and chalcone components, constitute the primary non-volatile elements in *B. balsamifera*. Sesquiterpene lactones (SLs), found in many Asteraceae plants, are among the compounds discovered (Pang et al. 2014). The same study identified three sesquiterpene lactones: blumealactone A, blumealactone B, and blumealactone C. Fujimoto et al. (1988) extracted these compounds from dried *B. balsamifera* leaves using 90% ethanol. Moreover, terpenoids are a common class of compounds in *B. balsamifera*, and their cytoskeleton types include monoterpenoids, sesquiterpenes, diterpenes, and triterpenes, among others (Wang et al. 2023). Sesquiterpene lactones, particularly, have garnered interest due to their cytotoxic properties and potential as agents against tumors. Clarkson's et al. (2004) toxicity index suggests the mildly harmful result may be due to the sesquiterpene lactones in *B.* balsamifera's ethanolic extract.

***Coleus amboinicus* (*oregano*)**

Arumugam et al. (2016) assert that *oregano*'s natural phytochemical components with their nutritional and medicinal properties, are important to the pharmaceutical industry. The literature review identified 76 volatile and 30 non-volatile compounds, including monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols, and aldehydes. *P. amboinicus* is rich in oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. Like *A. vulgaris* and *B. balsamifera*, it contains flavonoids and sesquiterpenes contributing to its cytotoxicity. It is particularly rich in phenolic monoterpenes such as thymol and carvacrol, which have various pharmacological properties. However, scientific validation of traditional uses is needed to authenticate novel bioactive compounds from *C. amboinicus*. While cytotoxicity assays like BSLA and TBA provide initial toxicity data, further testing in more complex biological systems, including animal models and human clinical trials, is necessary to determine its human toxicity.

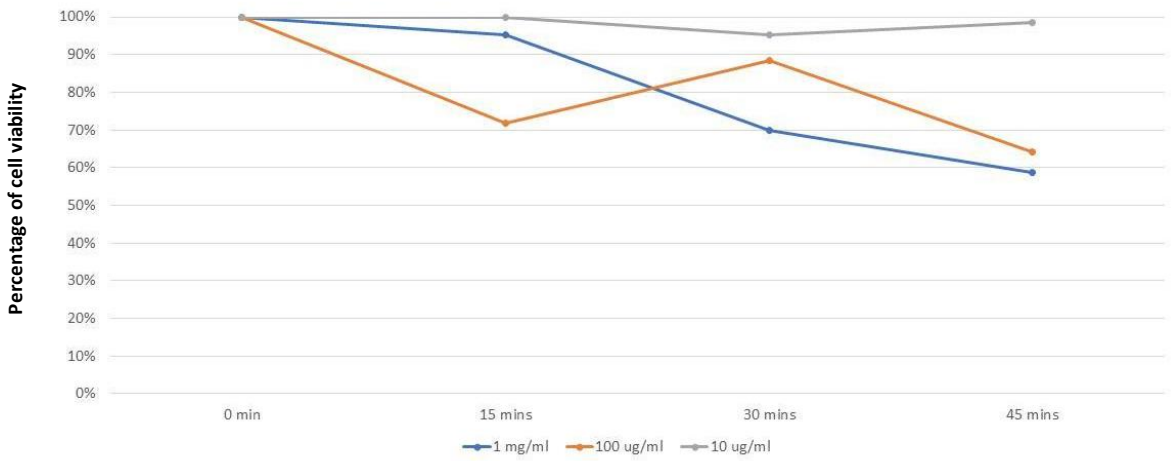

Figure 7. *Artemisia vulgaris* cell viability for different concentrations (1 mg/mL, 100 µg/mL, 10 µg/mL) under different exposure times (15, 30, and 45 minutes)

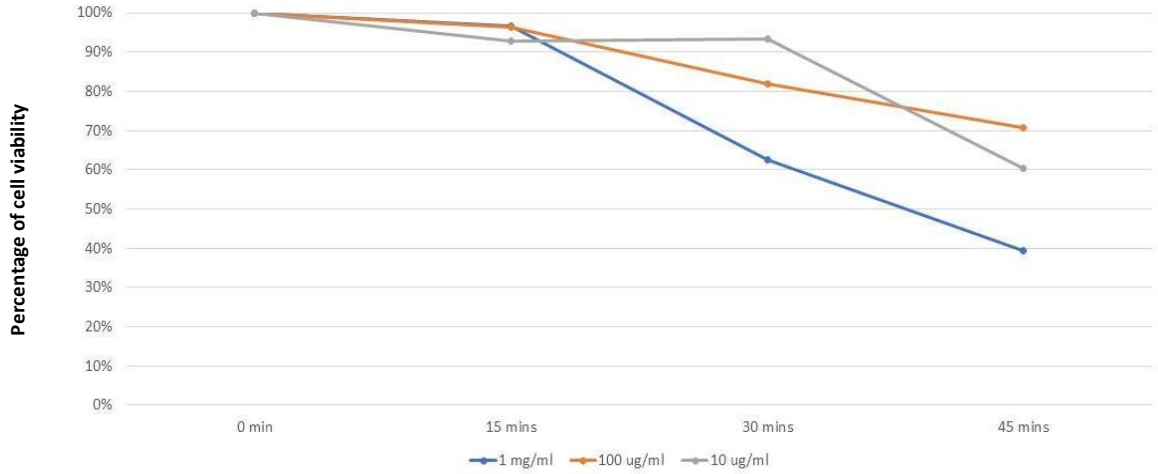

Figure 8. *Coleus amboinicus* cell viability for different concentrations (1 mg/mL, 100 µg/mL, 10 µg/mL) under different exposure times (15, 30, and 45 minutes)

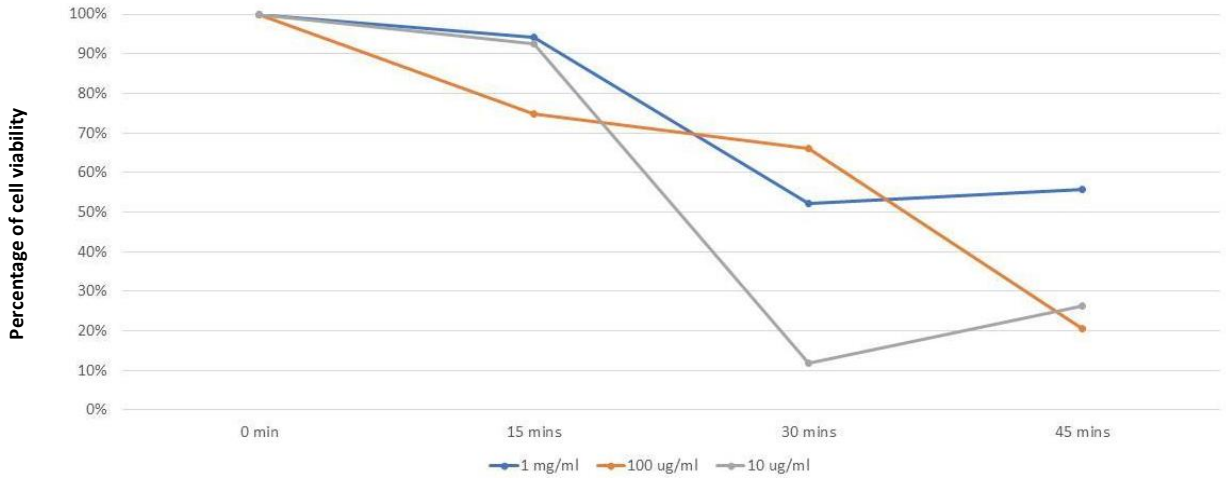

Figure 9. *Blumea balsamifera* cell viability for different concentrations (1 mg/mL, 100 µg/mL, 10 µg/mL) under different exposure times (15, 30, and 45 minutes)

Trypan Blue Assay

The assessment of the cytotoxic effects of *A. vulgaris* on cell viability at various concentrations and exposure durations unveiled several significant patterns as seen in Figure 7. During the 15-minute exposure period, the cell viability at the highest concentration of 1 mg/mL was 95.29%, indicating a consistently high level of viability. Nevertheless, when the concentration was set at 100 µg/mL, the viability of the cells decreased to 71.75%, suggesting a notable decrease. Notably, the lowest 10 µg/mL concentration could sustain full cell viability at 100%. These findings indicate that even brief exposure to moderate concentrations can negatively impact cell viability. However, cells appear more resilient when exposed to higher doses for shorter periods, showing minimal loss of viability.

There was a clear decrease in cell viability as the exposure time reached 30 minutes. With an elevated concentration of 1 mg/mL, the viability of cells decreased to 69.86%, indicating a rise in cytotoxicity after prolonged exposure. The cell viability was higher (88.35%) for the medium concentration (100 µg/mL) than for the 15-minute exposure. There are a couple of possibilities for this observation. It could indicate that cells adapt or suggest that the experimental conditions are being altered. At a concentration of 10 µg/mL, there was a minor decline in viability to 95.29%. This suggests that lower concentrations have a relatively minimal long-term impact.

During the 45-minute exposure period, the cytotoxic effects became more evident. Cell viability decreased to 58.68% at 1 mg/mL concentration and reduced to 64.29% at 100µg/mL. The results demonstrate a noticeable decrease in cell viability as the concentration and exposure time increase. Nevertheless, the 10 µg/mL concentration consistently exhibited a high cell viability of 98.5%, indicating that lower concentrations of A. vulgaris are considerably less harmful to cells even with prolonged exposure.

In 2009, a study by Emami et al. (2009) examined the anticancer effects of five different species of *Artemisia* on Hep2 and HepG2 cell lines. It was discovered that *A. vulgaris* can eliminate these cell lines due to the presence of sesquiterpene lactones, terpenoids, and flavonoids. A recent study by Jakovljević et al. (2020) discovered that *A. vulgaris* has cytotoxic effects attributed to its flavonoids and phenolic compounds. It was evident from the results that higher concentrations of 50, 100, and 250 µg/mL led to an increased presence of micronuclei in peripheral blood lymphocytes. Combining these compounds with mitomycin C resulted in cytotoxic effects while not significantly impacting the viability of human periodontal ligament stem cells. Essential oils, specifically the essential oil derived from the leaves, have been found to induce apoptosis in different cancer cell types while sparing normal cells. According to Saleh et al. (2014), this indicates that they may have the potential to serve as a novel class of anticancer medications.

As presented in Figure 8, the cells' viability has remained impressively high throughout the 15-minute exposure period for *C. amboinicus*, the cells' viability has remained impressively high, regardless of the concentrations. At the highest concentration of 1 mg/mL, the cell viability was 96.7%, whereas at 100 µg/mL, it was slightly lower at 96.25%. At a concentration of 10 µg/mL, there was a slight decrease in cell viability to 92.84%. The findings suggest that short-term exposure to *C. amboinicus* does not significantly impact cell viability even when exposed to higher concentrations; short-term exposure to *C. amboinicus* does not significantly impact cell viability. This implies that there is a relatively low immediate cytotoxic effect.

With an increase in exposure time to 30 minutes, a noticeable decline in cell viability became evident, especially at higher concentrations. At the highest concentration of 1 mg/mL, there was a notable decrease in cell viability to 62.5%, suggesting considerable cytotoxicity after prolonged exposure. The viability of the intermediate concentration (100 µg/mL) was reduced to 81.93%, although it remained higher than the highest concentration. Notably, the lowest concentration of 10 µg/mL exhibited a remarkable cell viability of 93.24%, comparable to the results observed during short-term exposure. It can be inferred that lower concentrations *of C. amboinicus* have a relatively minimal impact over a reasonably long duration.

During the 45-minute exposure period, the cytotoxic effects became even more evident. When the concentration was at 1 mg/mL, the cell viability dropped significantly to 39.37%, indicating a notable rise in cytotoxicity after prolonged exposure. The viability of the intermediate concentration (100 µg/mL) was significantly reduced to 70.74%. The cell viability decreased significantly even at the lowest concentration (10 µg/mL), reaching 60.33%. This suggests prolonged exposure to *C. amboinicus* at any concentration reduces cell viability, with a more pronounced effect observed at higher concentrations.

A study conducted by Hasibuan and Rosidah in 2017 found that increasing the concentration of extracts reduced cell viability. The presence of phenolic compounds, specifically carvacrol and thymol, in *C. amboinicus* is the reason for this. The compounds mentioned in the study have been found to impact cancer cells significantly. They promote apoptosis, disrupt cell membranes, and enhance cytotoxicity and ROS levels, increasing oxidative stress in cancer cells (Pinheiro et al. 2015). *C. amboinicus* also contains flavonoids and sesquiterpenes, contributing to its cytotoxic effects on cells (Arumugam et al. 2016).

ANOVA

Viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11069.914	2	5534.957	6.280	0.003
Within Groups	68744.653	78	881.342		
Total	79814.567	80			

Figure 10. ANOVA results for cell viability with three plant extracts

Figure 9 shows during the 15-minute exposure period, the cell viability remained consistently high for most concentrations. At the highest 1 mg/mL concentration, the cell viability was 94.14%, suggesting minimal immediate cytotoxic effects. The 100µg/mL concentration exhibited a significant decrease to 74.74%, whereas the lowest concentration of 10µg/mL demonstrated a high cell viability of 92.5%. These findings indicate that brief exposure to *B. balsamifera*, especially at lower concentrations, has a lesser impact on cells.

Nevertheless, a longer exposure time resulted in a significant reduction in cell viability. After 30 minutes, 1 mg/mL concentration caused a significant decrease in cell viability, which dropped to 52.15%. The concentration of 100µg/mL displayed a moderate decrease to 65.98%, whereas the lowest concentration of 10 µg/mL demonstrated a significant reduction in cell viability to 11.86%. It can be observed that the cytotoxic effects of *B. balsamifera* become more noticeable with prolonged exposure, particularly at lower concentrations. This could imply a threshold effect, where lower concentrations are more lethal over extended periods.

The 45-minute exposure period it provided additional evidence of the cytotoxic effects of *B. balsamifera*. The 1 mg/mL concentration exhibited a modest increase in cell viability, reaching 55.8%. This observation may suggest the presence of a cellular adaptation mechanism or experimental variability. The concentration of 100 µg/mL demonstrated a notable reduction in cell viability to 20.65%, while the lowest concentration of 10 µg/mL yielded a viability of 26.21%. The results of this study emphasize the significant cytotoxic effect that occurs with prolonged exposure, especially at intermediate and low concentrations. This finding suggests a complex relationship between the dose and response.

The cytotoxic effects of *B. balsamifera* are mainly attributed to its non-volatile constituents, specifically flavonoids. A study by Pang et al. (2014) and Tan et al. (2013) discovered combining ultrasound and 30% ethanol can effectively extract flavonoids such as blumeatin, velutin, and quercetin, resulting in a concentrated solution. Scientists recognized the medicinal properties of these flavonoids, including their capacity to induce cytotoxicity. In addition, the plant contains other non-volatile compounds, such as sterols and sesquiterpene lactones, which can potentially be used as antitumor agents (Fujimoto et al. 1988).

Figure 10 shows data on the viability percentages of three different plants: *A. vulgaris* (*damong maria*), *C. amboinicus* (*oregano*), and *B. balsamifera* (*sambong*). Viability, expressed as a percentage, reflects the health and vitality of these plants under certain conditions. The results indicate no statistically significant difference in viability between the plant groups ($F = 6.280$, $p = 0.003$). However, intriguing trends emerge upon closer examination of the mean viability percentages. *Damong maria* demonstrates the highest mean viability at 80.17%, followed by *oregano* at 73.87% and *sambong* at 52.83%. Though the overall difference is not significant, it's noteworthy that *sambong* exhibits substantially lower viability compared to the other two plants. The post-hoc tests, which provide additional insights, revealed that *sambong*'s viability was significantly lower than that of *damong maria* (mean difference = -27.34, $p = 0.003$) and *oregano* (mean difference = -21.04, $p = 0.029$) at a 0.05 significance level. Moreover, examining the data for each plant individually provides additional context. For *damong maria*, a trend shows a decrease in viability with increasing concentration, with the highest average viability seen at 10 ug/mL (94.71%) and the lowest at 1 mg/mL (69.69%). Similarly, *oregano* showed a slight decrease in viability at higher concentrations, whereas sambong showed variability over a wide range of concentrations without a clear trend.

In conclusion, this study highlighted the critical role of traditional healing practices in Indigenous communities' healthcare systems and advocated for ongoing research and collaboration to preserve these practices. The study looked at the pharmacological properties of plant extracts from *A. vulgaris*, *C. amboinicus*, and *B. balsamifera*. It showed that the cytotoxic effects changed depending on the concentration and time. Findings indicated significant cytotoxicity at higher concentrations and variable responses at lower concentrations, highlighting the importance of precise dosage optimization and exposure duration. The study further recommended the need for advanced assays to elucidate the mechanisms of cytotoxicity and suggested similar studies in other indigenous tribes to document and preserve their medicinal knowledge. This approach supported the sustainability of traditional practices and assisted in discovering new therapeutic agents, emphasizing integrating traditional knowledge with modern scientific methods in drug discovery.

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