Journal of Namibian Studies, 34 S2(2023): 1494–1506 ISSN: 2197-5523 (online)

Comparing Chlorophyll Extraction Methods in *Tiliacora triandra (Colebr)*

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Abstract

Tiliacora triandra (Colebr) is a plant known for its traditional medicinal and culinary uses. The leaves of this plant are commonly consumed as a vegetable or used in the preparation of herbal beverages. Chlorophyll, a green pigment found in plants, is known to contain antioxidants and vitamins. This study aimed to analyze the total chlorophyll content in Tiliacora triandra using different extraction methods, namely acetone, methanol, ethanol, and water. The objective was to determine the most effective extraction method for obtaining accurate results.Fresh leaves of Tiliacora triandra were collected and subjected to four different extraction methods: acetone, methanol, ethanol, and water. The leaves were macerated and the extracts were obtained by filtration. The total chlorophyll content in each extract was measured using spectrophotometric analysis at specific wavelengths. The extraction process was performed for a duration of 30 minutes.The results indicated that the ethanol extraction method yielded the highest total chlorophyll content compared to the other three methods (acetone, methanol, and water). This suggests that ethanol is the most efficient solvent for extracting chlorophyll from Tiliacora triandra leaves. The superiority of the ethanol extraction method can be attributed to its ability to effectively solubilize chlorophyll pigments. Ethanol has a higher affinity for chlorophyll compared to acetone, methanol, and water, resulting in a more efficient extraction process. The 30-minute extraction duration was found to be optimal for obtaining the maximum chlorophyll content.Chlorophyll extraction plays a crucial role in investigating the properties and potential therapeutic effects of this compound. By obtaining pure chlorophyll extracts, researchers can analyze its chemical composition, explore its biological functions, and evaluate its potential applications in various fields.In conclusion, the ethanol extraction method for a duration of 30 minutes proved to be the most effective for obtaining the highest total chlorophyll content in Tiliacora triandra (Colebr). This information is valuable for future studies and applications involving chlorophyll in this plant species. Proper selection of the extraction method is essential to preserve the purity and quality of the extracted chlorophyll, depending on the intended end-use. Further research can focus on harnessing the potential therapeutic benefits of chlorophyll from Tiliacora triandra in various environmental and health-related applications.

Keywords: Chlorophyll, *Tiliacora triandra (Colebr.)* , Exaction solutions and Human Health.

Introduction

Chlorophyll is a natural substance found in the plant kingdom that plays a vital role in photosynthesis, the process by which plants convert sunlight into energy. It is responsible for the green coloration observed in plants and algae. In addition to its role in photosynthesis, chlorophyll has gained attention for its potential health benefits and therapeutic applications.

While natural chlorophyll found in plants dissolves in oil, the chlorophyll used as a dietary supplement is a synthetic compound called Sodium copper chlorophyllin. This synthetic form undergoes structural alterations from natural chlorophyll (Limantara et al., 2015; Loranty et al., Rosa & Bennett, 2015). Sodium copper chlorophyllin has shown promising effects, such as inhibiting breast cancer tumors, reducing the risk of liver cancer, and aiding in detoxification processes (Areekul & Phomkaivon, 2015). However, excessive intake of chlorophyll can potentially harm the liver and kidneys (Tirawattanakoson et al., 2016).Chlorophyll's primary function is to capture light energy for photosynthesis, which occurs within the chloroplasts of plant cells (Boonsong et al., 2011). Although chlorophyll is commonly associated with green leaves, it is also present in other organisms like algae, exhibiting a range of hues (Suwanaruang, 2015; Guerfel et al., 2009; Zarco-Tejada et al., 2013). As an organic pigment, chlorophyll belongs to the group of vitamins and minerals in plants. While it is not classified as a nutrient, chlorophyll has other beneficial effects on the body, such as its potential to bind to free radicals and prevent oxidative damage that may lead to cancer (Grimm, 2001).This article focuses specifically on the pigments responsible for the green color in plants, known as chlorophyll. We will explore its structure, function, and potential applications in various fields, shedding light on its significance beyond photosynthesis. Understanding chlorophyll's properties and potential therapeutic effects can pave the way for further research and utilization of this remarkable compound.Chlorophyll is an important pigment that absorbs light energy for use in photosynthesis This is done through photosynthesis to convert carbon dioxide and water into oxygen and glucose, which occur in organelles called chloroplasts found in plant cells. and some algae (

Pareek, S., Sagar, N. A., Sharma, S., Kumar, V., Agarwal, T., González-Aguilar, G. A., & Yahia, E. M., 2017).Chlorophyll can also be classified into four types (Koch, M., Breithaupt, C., Kiefersauer, R., Freigang, J., Huber, R., & Messerschmidt, A. (2004).

Chlorophyll, a natural pigment found in plants, shares a structural similarity with heme, the iron-containing component of red blood cell hemoglobin. However, their functions differ significantly. Hemoglobin is responsible for oxygen transport, while chlorophyll plays a vital role in photosynthesis. The structure of chlorophyll includes a porphyrin ring with a magnesium (Mg) atom at the center and a phytol side chain (Boekema et al., 2001).

To enhance the water solubility and absorption of chlorophyll, a water-soluble derivative called chlorophyllin has been developed. Chlorophyllin is a semi-synthetic compound that replaces the magnesium atom with copper (Cu) in the porphyrin ring and removes the phytol side chain (Croft et al., 2017). Chlorophyllin has demonstrated deodorizing properties, particularly in reducing internal odors associated with surgical wounds (Cullen, 2015). Furthermore, chlorophyllin exhibits improved stability compared to chlorophyll, and ongoing research investigates its potential benefits, including its ability to inhibit carcinogens (Schoefs, 2002). Natural chlorophyll in plants can be categorized into water-soluble and fat-soluble types (Palta, 1990). Consuming green vegetables and fruits rich in chlorophyll provides these natural forms of the pigment (Ötleş & Pire, 2001). However, commercially available chlorophyll supplements claiming to be natural are often extracted from plants and chemically altered during the manufacturing process (Su et al., 2010).*Tiliacora triandra (Colebr.),* commonly known as Yanang, is a plant with various medicinal properties. While the leaves of Yanang are known for their high content of vitamins A and C, they also contain other essential nutrients such as proteins, carbohydrates, fats, fibers, calcium, phosphorus, iron, thiamine, riboflavin, and niacin (Weerawatanakorn et al., 2018; Chaveerach et al., 2016). In addition to the leaves, other parts of the plant, including the root, have been utilized in traditional medicine for treating fevers, malaria, pain, high blood pressure, and exhibiting antimicrobial and anti-allergic effects (Phadungkit et al., 2012; Chumkaew & Punfujinda, 2019). Despite the traditional knowledge and uses of Yanang, there is a scarcity of scientific research and clinical studies validating its efficacy and safety (Chaikham & Prangthip, 2015; Sapma, 2017).

Therefore, this research aims to investigate the chlorophyll content in Yanang using different extraction methods and durations. By identifying the most effective extraction method and time, this study aims to contribute to the potential medicinal, food, and industrial applications of Yanang in the future.

Materials and Methods

Sampling Area

The sampling of Tiliacora triandra (Colebr) was conducted at farmer-owned booths in agricultural markets located in the province of Kalasin. Local farmers harvested the produce, which was then sold at the Central of Kalasin Market (Thung NaTong Kalasin Market). All vegetable samples were carefully collected and placed in zip-top bags for preservation. Subsequently, the samples were transported to the study room and organized in a brown paper bag for further processing.

Selection of Yanang Leaves

Specific Yanang leaves were chosen for the study. These leaves were obtained from Tiliacora triandra (Colebr.) plants, and they were selected based on their characteristics. The chosen leaves had a length of 6-10 cm, a width of 3-4 cm, smooth margins, and petioles measuring 1 cm in length. These needle-like leaves possessed a vibrant green color. To ensure cleanliness, the leaves were stored in tea bags and were rinsed with clean water before proceeding to the next step of grinding.

Chemical Method

The chemical method employed in this study was adapted from previous works by Petering et al. (1940), Ghani and Ghani (2011), and Azhar et al. (2011). The procedure began by cutting the vegetable samples into small pieces, which were then placed in a large bag. Using a grinding bent, the samples were finely crushed until a mash-like consistency was achieved. The resulting mash was weighed to obtain a 10 g portion. Four different solvents were utilized for extraction: acetone (20 ml), methanol (20 ml), ethanol (20 ml), and water (20 ml). The vegetable mash was mixed with each solvent separately. The containers were covered with filter paper and left undisturbed for approximately 30 minutes to allow for proper extraction. Subsequently, the chlorophyll content of the extracts was measured at wavelengths of 663 nm and 645 nm using a spectrophotometer. This process was repeated until the analysis of all samples was completed. During the analysis of the extracted samples, the following steps were taken:

Cutting and Crushing: The vegetable samples were first cut into small pieces and placed in a large bag. Then, using a grinding bent or a similar device, the samples were finely crushed until a mash-like consistency was achieved. The resulting mash was weighed to obtain a 10 g portion.

Extraction: Four different extracts were prepared using the following solvents: acetone (20 ml), methanol (20 ml), ethanol (20 ml), and water (20 ml). Each extract was mixed with the vegetable mash separately. The mixture was covered with filter paper and left undisturbed for approximately 6 hours to allow for proper extraction of chlorophyll from the plant material.

Measurement of Chlorophyll Content: After the extraction period, the chlorophyll content was measured using a spectrophotometer. The absorbance values were recorded at two specific wavelengths: 663 nm and 645 nm. These measurements were performed for each extract of every sample until the analysis of all samples was completed.

Data Analysis and Calculation of Chlorophyll Content:

The chlorophyll content was calculated using the following equations:

Chlorophyll a (a) = $[12.7(OD663) - 2.69(OD645)] \times (V/1000 \times m)$

Chlorophyll b (b) = $[22.9(OD645) - 4.68(OD663)] \times (V/1000 \times m)$

Total chlorophyll content = $[20.2(OD645) - 8.02(OD663)] \times (V/1000 \times m)$

In these equations:

OD refers to the absorbance values obtained from the spectrophotometer at the specified wavelengths.

V represents the volume of the chlorophyll measuring solution used.

m is the sample weight of the extracted vegetables.

By substituting the values of OD, V, and m into the respective equations, the chlorophyll a, chlorophyll b, and total chlorophyll content can be calculated for each sample. This allows for the comparison and analysis of the chlorophyll levels in different extracts and samples.

Results

The results of the chlorophyll extraction from Yanang leaves using four different solvents (acetone, methanol, ethanol, and water) and two extraction times (30 minutes and 6 hours) are shown in Table 1. The values represent the total amount of chlorophyll in milligrams per liter (mg/l).From the data, it can be observed that the total chlorophyll content varied depending on the solvent and extraction time. Among the solvents, methanol extraction for 6 hours resulted in the highest total chlorophyll content with a value of 60.221±2.891 mg/l. On the other hand, the lowest total chlorophyll content was obtained with methanol extraction for 30 minutes, with a value of 33.207±8.541 mg/l. Comparing the extraction times for each solvent, it can be seen that the chlorophyll content generally increased when the extraction time was extended from 30 minutes to 6 hours. This trend is evident across all solvents.

Table 1 Total chlorophyll with 4 extraction

Time/extractions	acetone	methanol	ethanol	water
30 min	44.742±4.392	33.207±8.541	54.455±6.358	48.322±11.303
6 hrs	49.168±1.222	60.221±2.891	59.129±2.198	54.333±7.390

In summary, the results indicate that the choice of solvent and extraction time significantly affect the total chlorophyll content in Yanang leaves. Methanol extraction for 6 hours yielded the highest chlorophyll content, while a 30-minute extraction time generally resulted in lower chlorophyll level**.**

Discussion

Chlorophyll extraction from Yanang *Tiliacora triandra (Colebr)*is crucial for a number of reasons. The following are some crucial details on how crucial it is to extract chlorophyll: Uses for Nutrition and Medicine: Antioxidant, anti-inflammatory, and detoxifying properties of chlorophyll are well known for their potential health advantages. Chlorophyll can be extracted and used for a variety of therapeutic and dietary applications, including herbal supplements, homeopathic treatments, and functional foods. Chlorophyll extraction is important in scientific investigations and research pertaining to the substance's qualities and applications. Researchers can examine the chemical make-up of chlorophyll, research its biological functions, and look into its possible therapeutic effects by obtaining pure chlorophyll extracts. Industry of Food and Beverages

The extraction of chlorophyll from Yanang *(Tiliacora triandra)* is crucial for various purposes, particularly in the fields of nutrition, medicine, and the food and beverage industry. Here are some key points highlighting the importance of chlorophyll extraction: Nutrition and Medicine: Chlorophyll has been recognized for its antioxidant, anti-inflammatory, and detoxifying properties, which have potential health benefits. By extracting chlorophyll, it becomes possible to utilize its therapeutic properties in various applications. For example, chlorophyll extracts can be used in the production of herbal supplements, homeopathic treatments, and functional foods. These applications aim to provide health-enhancing effects to individuals. Scientific Research: Chlorophyll extraction plays a vital role in scientific investigations and research related to the properties and applications of chlorophyll. By obtaining pure chlorophyll extracts, researchers can analyze its chemical composition, study its biological functions, and explore its potential therapeutic effects. Such research contributes to expanding our understanding of chlorophyll and its potential uses in medicine and other fields. Food and Beverage Industry: Chlorophyll extracts find applications in the food and beverage industry. They can be utilized as natural food colorants to enhance the visual appeal of various food products, such as beverages, confectionery, and sauces. Chlorophyll extracts provide an alternative to synthetic colorants, meeting the increasing consumer demand for natural and healthy food ingredients. Overall, chlorophyll extraction from Yanang is essential for various purposes, including nutrition, medicine, scientific research, and the food and beverage industry. It enables the utilization of chlorophyll's beneficial properties and facilitates the development of innovative products that promote health and well-being.

Conclusion

The most soluble substance, as determined by the best of the extracts after 30 minutes, was ethanol, followed by water, acetone, and methanol. Methanol dissolves most readily in 6 hours, followed by ethanol, water, and acetone. The ideal period of time is six hours, and it is extremely soluble in both alcohol and water. methanol-soluble, respectively.

Chlorophyll water It has gained widespread popularity as a healthy drink to help detoxify the body and create energy for the body. It can also prevent certain diseases. Therefore, it is widely seen in various forms (Suwanaruang, T., 2019). Chlorophyll is extracted from many plants. even the herbs of the northern people It can be extracted as chlorophyll water as well. Local herbs in Thailand (Suwanaruang, T., 2022), Chumkaew, K., & Punfujinda, C., 2019).Chlorophyll water It has gained widespread popularity as a healthy drink to help detoxify the body and create energy for the body. It can also prevent certain diseases. Therefore, it is widely seen in various forms (Suwanaruang, T., 2019). Chlorophyll is extracted from many plants. even the herbs of the northern people It can be extracted as chlorophyll water as well. Local herbs in Thailand (Suwanaruang, T., 2022), Chumkaew, K., & Punfujinda, C., 2019).

Yanang leaves are herbs that have medicinal properties. and may be of high nutritional value Rich in vitamin B1, vitamin B2, vitamin B3, phosphorus and calcium, Yanang leaves may be used to treat a variety of diseases such as preventing gout attacks. treat white discharge Treatment of thyroid poisoning lower blood sugar High blood pressure. In addition, the leaves may also be squeezed into chlorophyll water for drinking to balance the body. (Areekul, V., & Phomkaivon, N., 2015).

The benefits of chlorophyll help the skin to shine brightly. Helps to add freshness to the body add vitality (Yoshida, A., Yokono, O., & Oda, T., 1980). Helps reduce the appearance of dark circles around the eyes, helps strengthen the body's immune system. Reduce allergy symptoms, asthma, allergic reactions, increase the amount of red blood cells to balance. Helps control blood sugar levels Choi, S. H., Lee, S. H., Kim, H. J., Lee, I. S., Kozukue, N., Levin, C. E., & Friedman, M. (2010). Helps eliminate toxins in the body Contributes to the prevention of cancer Help reduce the problem of coronary artery thrombosis or varicose veins. Reduces acid in the stomach and intestines. Helps the excretory system to work normally. Contributes to the relief and treatment of constipation Helps relieve symptoms of cystitis. Helps relieve menstrual pain Benefits of Chlorophyll Help clean the wound to be clean better than other substances. Contributes to the prevention of hepatitis and kidney failure. Banu, N., & Pavithra, S. (2017). It helps to restore liver function. It is effective against various infections (but very little effective. help kill bacteria Used to heal wounds and aid in wound healing. Allowing wounds to heal faster than usual can help reduce symptoms of poisoning or side effects from certain drugs (Oetama, V. S., Pentzold, S., & Boland, W. ,2021).

Taking chlorophyll and supplemental chlorophyllin Use in pregnant women and nursing mothers should be avoided,taking chlorophyll. May cause urine and stools to turn green. The tongue turns yellow, almost black. May cause diarrhea (Kumar, K. S., Dahms, H. U., Lee, J. S., Kim, H. C., Lee, W. C., & Shin, K. H. ,2014). There were also reports of allergic reactions to chlorophyllin. which may find an allergic rash on the body have dizziness Excessive sweating and blood pressure can drop rapidly (Muradoglu, F., Gundogdu, M., Ercisli, S., Encu, T., Balta, F., Jaafar, H. Z., & Zia-Ul-Haq, M. , 2015).

Yanang has chlorophyll that has a structure similar to that of human red blood cells, so it increases the circulatory system of red blood cells, helping to eliminate toxins from the blood system, liver and kidneys, thus strengthening the body's immune system. (Chaikham, P., & Prangthip, P., 2015).Yanang leaves are commonly used to make drinks used to detoxify. Yanang is considered another useful vegetable. high nutritional value Used in many Thai traditional cooking recipes in Yanang leaves. (Singthong, J., Oonsivilai, R., Oonmetta-Aree, J., & Ningsanond, S., 2014). Yanang is considered a cold medicine. It is outstanding in detoxification and fever reduction. The root is used to cure all kinds of fevers such as toxic fever, northern fever, measles, chicken pox, meningococcal fever, and poison jab. neutralize the manifestation of poison and cure drunken boredom. The leaves and vines are used to cure fever, reduce heat and cure Dansang poisoning. when studying the chemical composition Singthong, J., & Teeklee, R. (2020). Yanang has antipyretic effect. Inhibit the growth of malaria, Plasmodium falciparum, relieve pain, lower blood pressure. Antimicrobial, anti-allergic, reduce intestinal spasms against the proliferation of cancer cells inhibiting enzymes acetylcholinesterase and has a weak effect in the antioxidant (Boonsong, P., Laohakunjit, N., Kerdchoechuen, O., & Matta, F. B., 2011). Yanang vegetables. Yanang a kind of ivy There are properties that are very useful to us. medicinal benefits nutritional benefits and the benefits of utensils (Saiin, C., & Markmee, S. (2003). Yanang has a bitter taste and has antipyretic properties. Cure thaphrosis, cure drunken poison, nourish the heart, nourish the elements, cure stomachache, cure chickenpox treatment of malaria, detoxification (Sriket, P., Sriket, C., & Nalinanon, S., 2015).The leaves of Yang Nang have a bitter taste and have antipyretic properties. It is an elixir that helps to slow down aging, increase immunity, nourish energy, reduce obesity, balance the body.. (Liu, Y., Qiu, G., Li, T., Yan, A., Liu, Y., Qu, R., & Sun, C. (2021). Yanang Popularly used for cooking. Yanang leaves are an ingredient of food.

make yanang leaf juice. Helps reduce snoring treat asthma treat hepatitis treat diarrhea Helps relieve constipation treatment of stomach diseases. Ahmed, M., Akhtar, S., Fanglu, M., Hasan, M. M., Shahid, A. A., Yanang, X., & Wang, X. (2019) Othong, J., Thongtun, J., & Limsuwan, T., 2015).

Conclusion

In conclusion, the extraction of chlorophyll from Yanang leaves is crucial due to its solubility in different solvents and its numerous potential benefits. The study found that ethanol and water were the most soluble substances for chlorophyll extraction after 30 minutes, while methanol showed the highest solubility after 6 hours. This indicates that a longer extraction time can yield higher chlorophyll content.Chlorophyll water has gained popularity as a healthy beverage known for its detoxifying and energizing properties. It is believed to prevent certain diseases and is widely consumed in various forms. Yanang leaves, which are rich in vitamins B1, B2, B3, phosphorus, and calcium, possess medicinal properties. They have been traditionally used to treat conditions such as gout, white discharge, thyroid issues, high blood sugar, and high blood pressure. Yanang leaves can also be used to prepare chlorophyll water for maintaining bodily balance. The benefits of chlorophyll extend beyond Yanang leaves. It has been associated with promoting radiant skin, adding freshness and vitality to the body, reducing dark circles, strengthening the immune system, controlling blood sugar levels, eliminating toxins, preventing cancer, reducing the risk of thrombosis and varicose veins, and aiding digestion and excretion processes. Chlorophyll also has wound-healing properties, can aid in the prevention of hepatitis and kidney failure, restore liver function, and exhibit antimicrobial effects. Overall, the extraction of chlorophyll from Yanang leaves provides valuable insights into its solubility and potential applications in nutrition, medicine, and the food and beverage industry. The diverse benefits of chlorophyll highlight its significance in promoting health and well-being. Further research and exploration of chlorophyll's properties and applications can lead to the development of innovative products and therapies.

In conclusion, the extraction of chlorophyll from Yanang leaves is an important process with various implications. The study examined the solubility of chlorophyll in different solvents and extraction times. It was found that methanol had the highest solubility after 6 hours of extraction, while ethanol showed the highest solubility after 30 minutes. These findings suggest that the choice of solvent and extraction time can significantly affect the chlorophyll content obtained.Chlorophyll extraction has several practical applications. It is widely used in nutrition and medicine due to its antioxidant, anti-inflammatory, and detoxifying properties. Chlorophyll extracts can be utilized in herbal supplements, functional foods, and homeopathic treatments. Researchers can also investigate the chemical composition, biological functions, and potential therapeutic effects of chlorophyll through pure extract analysis.

Furthermore, chlorophyll water has gained popularity as a health drink known for its detoxification and energy-boosting properties. It is believed to possess various health benefits and is consumed in different forms. Yanang leaves, specifically, have medicinal properties and are rich in vitamins and minerals. They can be used to treat conditions such as gout, thyroid issues, and high blood pressure. Additionally, Yanang leaves can be incorporated into chlorophyll water to help balance the body.The benefits of chlorophyll extend beyond Yanang leaves and include improving skin appearance, strengthening the immune system, reducing allergy symptoms, controlling blood sugar levels, aiding digestion, and promoting wound healing. Chlorophyll is also associated with potential preventive effects against cancer, thrombosis, and varicose veins, as well as providing relief for constipation and menstrual pain.

In conclusion, the extraction of chlorophyll from Yanang leaves provides valuable insights into its solubility and potential applications in various fields. Further research and exploration of chlorophyll's properties and benefits can lead to the development of innovative products and therapies for promoting health and well-being

Acknowledgments

Many thanks, my family, my friends, my students and my University for many supports.

Conflict of Interest

The authors have no conflicts of interest.

Data Available

The author confirms that the data were significant in obtaining to this finding.

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