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Research

Phytochemical Constituents and Toxicity Analysis of Ethanolic Ketapang (*Terminalia catappa* L.) Leaf Extract

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ABSTRACT

Terminalia catappa L., which is also known as Indian almond, tropical almond, and ketapang, belongs to the family of Combretaceae and it forms layers of canopy, which provides shade to locals. The parts of the plant such as bark, fruit, leaf, rhizomes, and roots have been traditionally used in folk medicines for several treatment purposes, demonstrating its numerous biological activities. The current study evaluated phytochemical constituents in its leaf responsible for its biology activities and toxicity analysis by brine shrimp lethality test for ethanolic leaf extract of *T. catappa* L. (EKLE) to set a safe limit for future applications in studies. Phytochemical compounds such as squalene, phytol, DL-α-tocopherol, β-sitosterol, stigmasterol, α-amyrin, and β-amyrin were identified in EKLE through GC-MS analysis, which is believed to contribute to its biology activities such as antibacterial. This is the first time to report β-sitosterol in the leaf of *T. catappa* L., though previous studies have reported in the bark of the tree and other parts of its genus. This is the first time to identify β-amyrin in this tree. The LC_{50} value in the brine shrimp assay was above 100 µg/mL, suggesting the extract is biologically safe and non-toxic for humans. However, the application of the extract shall not be more than 11.61 mg/mL.

Key words: Amyrin, ketapang, phytochemical, shrimp, squalene, toxicity

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INTRODUCTION

Terminalia catappa L. which is also known as the Indian almond tree belongs to the family of Combretaceae and it is indigenous to Southeast Asia, where the tree is valued as folklore medicines as well as ornamental (Chansue & Assawawongkasem, 2008). Though the leaves are green in color initially, they'll turn to red, then yellow, and lastly to copper brown according to seasonal change before shedding off (Marjenah & Putri, 2017).

There are several biology activities reported on this tree, which covers a wide range of spectrum. Nampoothiri *et al*. (2011) reported the anti-diabetic function of *T. catappa* L. extract as it has significant α-amylase and α-glucosidase inhibitory actions because they reduce the concentration of blood glucose levels by increasing the production of insulin levels. Furthermore, the hydrophilic leaf extract of the tree protects human skin fibroblasts from photoaging upon exposure to type B ultraviolet (UVB) radiation, demonstrating anti-aging bioactivity (Silalahi, 2022).

Terminalia catappa L. has several phytochemical constituents such as alkaloids, amino acids, flavonoids, glycosides, phenolic compounds, saponins, steroids, sugars, and tannins which contribute to its biological activities (Poongulali & Sundararaman, 2016; Salares & Balala, 2018). The leaves of this plant have been reported to have antimalarial activity contributed by its alkaloid content (Muhammad & Mudi, 2011). Besides that, the flavonoids found in the leaves of this tree are considered responsible for their antimicrobial functionality against a wide range of bacteria (Allyn *et al*., 2018). However, the concentration

needed to achieve all the functionality varies, and safety concern arises for consumers for applications of the extract concentration. The current study aims to identify the physicochemical properties of dried green leaf powder of *T. catappa* L., identify the phytochemical components of ethanolic *T. catappa* L. leaf extract, and determine its toxicity by brine shrimp lethality assay.

MATERIALS AND METHODS

Plant sampling and extraction

Green leaves of *ketapang* were collected as samples for the current study from Taman Pertanian, Bukit Ekspo, Universiti Putra Malaysia (UPM), Selangor, Malaysia. The leaves were identified and a voucher specimen (SK3336/18) was deposited by a botanist from the Biodiversity Unit, Institute of Bioscience, UPM. Then, the leaves were washed under running tap water, followed by cleaning with distilled water. Next, the samples were dried in an oven drier (SMA-112, Smoke Master, Toda, Japan) at a temperature of 50 °C for two days, continuously. Dried samples were collected and checked for moisture content to ensure the desired drying level was achieved. Then, the dried samples were grounded by using a dry blender (Panasonic MX-GM1011, Osaka, Japan) until fine powder was obtained. The dried sample powder was then stored in a chiller of temperature 4 °C throughout the study.

Later, maceration was carried out by using 100 g of dried powder of the sample with 400 mL of 95% (v/v) ethanol with a ratio of 1:4. Then, it was stored for a week at room temperature, accompanied by occasional shaking. Whatman filter paper No.1 (Whatman International Ltd., Middlesex, England) was used to filter out the leaf extract, followed by concentration of the extract by rotary vacuum evaporator (BUCHI Rotavapor R-200, Flawil, Switzerland) for 3 to 4 h at the temperature of 50 °C with speed of 150 r.p.m. The obtained crude extract was then stored in the chiller at a temperature of 4 °C.

Characterization of dried *T. catappa* L. powder

Physicochemical analysis for the dried fine powder of *T. catappa* L. leaf was carried out to obtain its color, moisture content, pH, and water activity. Color analysis was carried out using a colorimeter (CR-400, Konica Minolta, Japan, moisture content was carried out using a moisture analyzer (MX-50, A&D Company, Japan), pH was determined using benchtop lab pH meter (3510, Jenway, Malaysia) and water activity of the powder was determined using benchtop lab water activity meter (AquaLab Pre, METER Group, United States). All the analyses were done in triplicates.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was carried out to identify volatile compounds present in the *T. catappa* L. extract. The extract was dissolved in HPLC-grade methanol to yield a concentration of 10 mg/mL. The GC-MS protocol used in this study is an in-house method. The analysis was performed in a QP2010 Ultra gas chromatograph-mass spectrometer (Shimadzu Corporation, Kyoto, Japan), which had an electron multiplier detector. The analyzer used a column that was Rxi-5ms with a length of 30.0 m, internal diameter of 0.25 mm, and film thickness of 0.25 µm. Helium was used as carrier gas with a flow rate of 0.80 mL/min for the column. The operating conditions of the analyzer include an initial temperature of 50 °C for the column oven, with an increase rate of 3 °C/min till up to 300 °C at the end, where the hold time was 10 min. The injection temperature and ion-source temperature were both 250 °C and 200 °C, respectively. The peaks for the extract of *T. catappa* L. were determined by calculating and comparing their similarity index, retention indices (RI), and mass fragment patterns with the standard spectra provided in the Shimadzu GC-MS NIST / Wiley library. The data were also compared with previously published studies about the volatile compounds found in *T. catappa* L. leaf extract.

Brine shrimp lethality assay

The brine shrimp lethality assay was performed to determine the cytotoxic activity of the *T. catappa* L. methanolic extract as described by Juwitaningsih *et al*. (2021) with some slight modifications. Brine shrimp (Artemia salina) eggs (JBL Artemio Mix, Germany) were purchased from an aquatic shop, located in Bangi, Selangor, Malaysia. A spatula of shrimp eggs was allowed to hatch in artificially prepared seawater that was well aerated with the aid of an air pump. The nauplii were hatched within 24 h. The nauplii were separated from their eggs. The ethanolic extract was dissolved in 10% DMSO to obtain 100 mg/mL and the solution was further diluted in seawater to obtain 20 mg/mL stock solutions. The diluted extract was then added to the tubes containing 20 live brine shrimp nauplii in 20 mL of seawater, yielding solutions of varying extract concentration (0.63, 1.25, 2.50, 5.00, 10.0, & 20 µg/mL). The number of surviving shrimps nauplii in each concentration was counted for every hour till 24 h. Potassium dichromate (Merck Millipore, Darmstadt, Germany) was used as the positive control in this bioassay while the highest concentration of DMSO was used as the negative control. The percentage of mortality of the brine shrimp nauplii for *T. catappa* L. extract concentration and the controls were calculated. The logarithm of concentration versus the percentage of mortality was plotted using Microsoft Excel (version 2019, New York, United States), and a regression equation was obtained. The value of

LC_{50} was calculated from the equation.

RESULTS AND DISCUSSION

Characterization of dried *T. catappa* L. powder

The color, moisture content, pH, and water activity of dried *T. catappa* L. leaf powder are summarized in Table 1. The data are presented in mean ± Standard Error of the Mean (SEM).

L* stands for luminance, where this component represents the lightness or brightness of a color. It ranges from 0 (black) to 100 (white), therefore, a higher L value indicates a lighter color, while a lower value indicates a darker color. The L* value reading was 40.18 ± 0.08 which showed the powder was slightly dark in color. The a* component represents a color in the green-red axis. Positive values (a* > 0) indicate redness, while negative values (a* < 0) indicate greenness. The a* value obtained for the powder is -0.07 ± 0.04, showing green color characteristics. The b* component represents the color on the blue-yellow axis. Positive values (b* > 0) indicate yellowness, while negative values (b* < 0) indicate blueness. The b* value obtained for the powder is 18.74 ± 0.04, showing a hint of yellowness (International Commission on Illumination, 2018). This is by a report by Kadam *et* al. (2011), where *T. catappa* L. leaf is described as dark green color. The moisture content of the powder was 7.12 ± 0.02%. This is somewhat comparable to the moisture content of *T. catappa* L. dried leaf (8.30 ± 2.35%) in a proximate study by Okpako *et al*. (2017). The low percentage of moisture content obtained after consistent weight upon drying was important to process the leaves into fine powder. Besides that, the low moisture sample will have a longer shelf life of powder in terms of quality throughout the storage period.

The water activity obtained for the powder was 0.54 ± 0., 01. Water activity is a crucial physicochemical factor in dried products, where if the value is lower than 0.60 upon drying, the product does not even need to be stored in a chiller (4 °C) to prevent microbial spoilage. This is because the minimum water activity needed for bacterial growth is 0.91, yeast growth is 0.85, and mold growth is 0.80 (Tapia *et al*., 2020). Therefore, the powdered sample is safe to be stored for a long period in powder form. The pH of the dried powder was 6.86 ± 0.00, which shows it was only slightly acidic; almost near to neutral. This is by a study by Bryan (2016), where dried *T. catappa* L. leaves were used in water treatment as a natural coagulant to remove turbidity. The pH of the treated water did not have a significant difference when different concentrations of *T. catappa* L. leaf were added. The slight acidity is possibly caused by the acidic bioactive components in the leaf such as tannins (Wang *et al*., 2020).

Yield of *T. catappa* L. leaf extract

Dried leaf extraction of *T. catappa* L. was performed by using 95% ethanol, yielding liquid crude extracts of dark green in color and high in viscosity. The yields of extraction are tabulated in Table 2.

The average yield obtained in this study was 13.26 ± 0.00%, from 100 g of dried and powdered samples. At the end of the extraction process, the extraction temperature of the rotary evaporator was set to 85 °C for 2 × 30 s. It is expected for ethanol present to completely evaporate as the boiling temperature of ethanol is 78 °C. Therefore, the *T. catappa* L. extract prepared is free from ethanol. The yield percentage obtained in this study is higher than studies done for *T. catappa* L. leaves by Yunita *et al*. (2021) and Chansue and Assawawongkasem (2007), where the yield of extracts was 5.20% with 90% ethanol and 12.9% with 95% ethanol as solvent, respectively. Based on several studies, *T. catappa* L. leaves are expected to have tannins. In a study done for optimization of tannin extraction, it was found that 95% ethanol solvent yielded the most tannin, compared to other extraction solvents such as water and 50% ethanol. As tannins are water-soluble polyphenols, they can result in impurities, mixing with other water-soluble components (Baldosano *et al*., 2015). Ethanol is one of the common organic solvents used in the extraction of leaves for studies related to food applications. Though methanol has a higher polarity, ethanol application in the food industry for extraction is considered safer than methanol, even though both are polar alcohols.

This is because methanol is toxic to the human body as it is metabolized by alcohol dehydrogenase, forming toxic compounds such as formaldehyde, which is later oxidized into formic acid (Ashurst & Nappe, 2018; Awad *et al*., 2021). Ethanol can be considered as the appropriate solvent to extract phenols, polyphenols, and any bioactive compounds from plants as it has a good polarity and is generally safe for human consumption (Altemimi *et al*., 2017). The extraction method used in this study was maceration as aforementioned. Seven days of maceration were carried out for the sample, has increased the chance of a good yield. In a study done on the extraction of *T. catappa* L. leaves to obtain a high yield of tannin, different numbers of days (2, 4, & 6) were used to study the yield of tannin alone and it was found day 6 was the most effective maceration period, concluding, longer maceration period, gives a better yield (Pramudita *et al*., 2018). Therefore, a good yield was obtained as this maceration allowed sufficient time for the ethanol to penetrate by molecular diffusion to solubilize them through the cell wall of *T. catappa* L. leaf powder as it provides a good surface area (Rasul, 2018).

Phytochemical components by GC-MS

In total, 10 peaks were identified in the GC-MS chromatogram performed on crude ethanolic extract of *T. catappa* L. leaf as shown in Figure 1. Table 3 summarises the identified volatile components found in this assay by calculating and comparing their similarity index, retention indices (RI), and mass fragment patterns with the standard spectra provided in the Shimadzu GC-MS NIST / Wiley library. Leaf extract of *T. catappa* L. is known to contain a diverse range of phytochemicals but many of these compounds might be present in trace amounts (Dwevedi *et al*., 2016). Therefore, a higher concentration (10 mg/mL), was used to ensure that a sufficient amount of these compounds would be present in the injection to enhance their detectability and quantification.

Table 1. Physicochemical analysis of dried *T. catappa* L. leaf powder

Properties	Readings
Moisture content	7.12 ± 0.02 %
pH**	6.86 ± 0.00
Water activity	0.54 ± 0.01
L*	40.18 ± 0.08
a*	-0.07 ± 0.04
b*	18.74 ± 0.04

#Footnote: ** in solution

Table 2. Yield of *T. catappa* L. leaf extract

Weight of sample (g)	Extract yield (g)
100.00 ± 0.00	13.26 ± 0.00

#Footnote: Data is presented in average ± standard deviation

The most abundant compound was DL-α-tocopherol (28.67%) (Peak no. 6), followed by phytol (23.30%) (Peak no. 4), squalene (14.83%) (Peak no. 5), β-sitosterol (13.92%) (Peak no. 8), β-amyrin (5.91%) (Peak no. 9) and α-amyrin (5.65%) (Peak no. 10). The remaining four compounds (ethyl α-D-glucopyranoside, neophytadiene, phytol acetate and stigmasterol) were present by the amount of less than 5%. To the best of our knowledge, this is the first time identifying β-sitosterol and β-amyrin in the leaf extract of *T. catappa* L. However, β-sitosterol has been identified in the bark of the *T. catappa* L. extract and other parts of its genus (Mohale *et al*., 2009; Das *et al*., 2020).

In a study by Anwar *et al*. (2022), β-sitosterol was isolated from *Kalanchoe tomentosa* leaves and tested for antibacterial activities against *Staphylococcus aureus* and *Klebsiella pneumoniae*, where the MIC values were 7.81 and 31.25 µg/mL, respectively, showing growth inhibition effect. Similarly, in another study, β-sitosterol was isolated from *Odontonema strictum* leaves to study antibacterial properties, in which, the component was tested against *S. aureus*. MIC and MBC were determined as 1.240 mg/mL and 2.208 mg/mL, respectively, which was about 3 times lower than crude extract to obtain the same outcome (Luhata & Usuki, 2021). These studies show the presence of β-sitosterol in the current extract makes it a potential antibacterial agent. In a study by Abdel-Raouf *et al*. (2013), β-amyrin was isolated from *Laurencia microcladia* (an alga) to learn its antibacterial activity against *S. aureus* and *Salmonella* Typhi. The MIC obtained for both of the test organisms was 2.5 mg/mL, showing the antibacterial property of the compound. Similarly, in a study by Rivero-Cruz *et al*. (2009), β-amyrin was one of eight compounds (β-amyrin, betulin, betulinic acid, oleanolic acid, quercetin, (-)-epicatechin, gallic acid, and β-sytosterol) isolated from *Byrsonima crassifolia* to study against 12 bacteria. MIC was performed and it was found that β-amyrin, gallic acid, and oleanolic acid were the only compounds that inhibited the growth of the bacteria among all the eight isolated compounds. Based on these findings of β-amyrin and β-sitosterol on antimicrobial properties, one of the functional properties of *T. catappa* L. leaf extract is antibacterial.

The volatile components found in GC-MS include sugar, terpenoid, diterpene, triterpene, phytosterol, sterol, and vitamins. All the compounds have been characterized and reported activities from previous studies are listed in Table 3. In a study by Inoue *et al*. (2005), a time-kill assay was performed by measuring K+ leakage from *S. aureus*, in which, phytol was one of the three diterpenes tested to learn the effects. It was found that the growth of *S. aureus* was inhibited by 0.15 µg/mL of the diterpene by damaging its cell membrane which proves the antibacterial functionality of phytol. In another study by Lee *et al*. (2016), the mechanism of phytol in antibacterial activity was assayed in *Pseudomonas aeruginosa* and it was found that phytol is capable of inducing oxidative stress in test organisms.

Neophytadiene is a diterpene hydrocarbon that has several biological activities such as antipyretic, anti-inflammatory, antimicrobial, and antioxidant, making the extract impactful from its presence in the leaf (Raman *et al*., 2012). In a recent study, neophytadiene was explored by *in vivo* neuropharmacological effects, and found that the compound has anxiolytic-like and anticonvulsant activities (Gonzalez-Rivera *et al*., 2023). Therefore, the extract could be studied further in terms of

its potential and applications in several sectors, including the pharmaceutical, food, and cosmetic industries. Squalene is a well-established triterpenoid that is being extracted from plant sources and

Table 3. Identification of phytochemical compounds in *T. catappa* L. leaf extract by using GC-MS

Peak No.	Constituent	Classification	Reported Activity	References
1	Ethyl α-D-glucopyranoside	Sugar	Antimicrobial	Abirami *et al*. (2021) Raman *et al*. (2012)
2	Neophytadiene	Diterpene	Antipyretic, anti-inflammatory, antioxidant, and antimicrobial	
3	Phytol acetate	Terpenoid	Antimicrobial, Anticancer	Sermakkani & Thangapandian (2012)
4	Phytol	Phytosterol	Antioxidant,	Inoue *et al*. (2005)
5	Squalene	Triterpenoid	Cardioprotector, antioxidant, antimicrobial, anticancer and detoxifier	Dmitrieva *et al*. (2022); Lozano-Grande *et al*. (2018)
6	DL-α-tocopherol	Vitamin	Antioxidant,	Bidossi *et al*. (2017)
7	Stigmasterol	Sterol	Anticancer, antitumor, antidiabetic and antimicrobial	Bakrim *et al*. (2022); Zhang *et al*. (2022); Poulose *et al*. (2021); Mailafiya *et al*. (2018)
, 8	β-sitosterol	Sterol	Antimicrobial, antioxidant	Luhata & Usuki (2021)
9	β-amyrin	Triterpenoids	Antibacterial, Antioxidant, Anticancer, Anti-inflammatory	Melo *et al*. (2011)
10	α-amyrin	Triterpenoids	Anti-inflammatory, Antioxidant, Antibacterial	Melo *et al*. (2011) Cardoso *et al*. (2020) Abdel-Raouf *et al*. (2015)

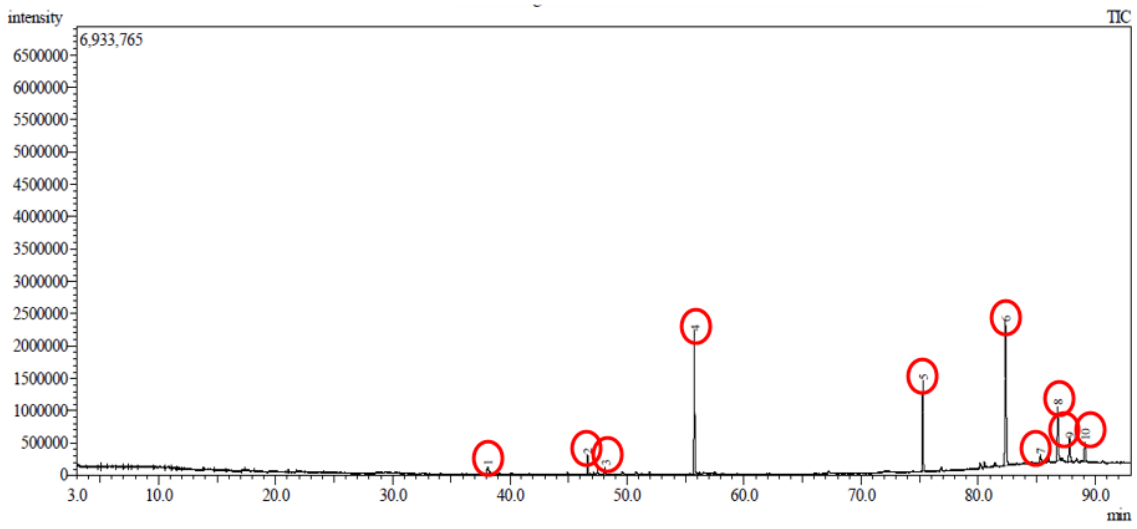

Fig. 1. GC-MS of phytochemical compounds of *T. catappa* L. leaf extract.

sold as a supplement due to its bioactive properties such as cardioprotector, antioxidant, antimicrobial, anticancer, and detoxifier (Lozano-Grande *et al*., 2018). The hunt for a squalene source and optimization in its extraction method is still an ongoing endeavor, making the *T. catappa* L. leaf highly valuable.

Stigmasterol is a plant sterol, typically found in herbaceous plants, belonging to tetracyclic triterpenes. The compound has a high demand and is currently studied *in vitro* and *in vivo* due to its abundant biological activities, especially in terms of anticancer and antitumor for its mechanisms and pathways (Bakrim *et al*., 2022; Zhang *et al*., 2022). In a recent study by Zhao *et al*. (2021), stigmasterol was found to cause apoptosis in gastric cancer cells, making a mark in studies of oncology. This is relatable to *T. catappa* leaf extract, where several studies have revealed the extract's efficiency in reducing the proliferation of cancer cells significantly (Shanehbandi *et al*., 2021; Zarredar *et al*., 2021). Therefore, stigmasterol could be one of the possible contributors to the anticancer property of the leaf extract. Apart from being an anticancer, stigmasterol is being studied for application and inclusion in food developments for its other well-established biological activities as well. One of them will be a study by Poulose *et al*. (2021), where the compound is extracted from seaweed and formulated into functional biscuits due to its antidiabetic functionality. Rats were used as study organisms in their research and sugar level was found to be reduced. Leaf extract of *T. catappa* L. has been reported to contain anti-diabetic properties by a few studies. In a study using Wistar albino rats and mice as study organisms, the pancreas of the test subjects was clinically damaged with alloxan. The leaf extract was observed to regenerate β-cells, revealing antidiabetic activity of the leaf extract (Ahmed *et al*., 2005). Therefore, stigmasterol could be one of the potential contributors to the anti-diabetic properties of the leaf extract.

Cytotoxicity of the extract by brine shrimp lethality assay

Herbal plants are rich in biological activities and have been used for centuries in folklore medicine to treat many health issues around the world. However, herbal plants can cause toxicity such as nephrotoxicity due to exposed chemicals, toxins, heavy metals, and even due to contaminants that can severely affect renal (Asif, 2012). Therefore, toxicity assessment is a crucial part of the studies of plant extracts if it is related to application in human food, medicine, or treatments. The brine shrimp lethality assay is vital to evaluate the safety and effectiveness of herbal plants by presenting the researcher with an early understanding of the toxicity of the chosen plant sample for studies.

Linear equation, y = 32.27x + 15.635 was generated from the graph shown in Figure 2. LC_{50} was calculated from the equation as shown below.

Let y be 50 as the LC_{50} represents the lethal concentration of 50%, where the y-axis represents mortality concentration.

$y = 32.27x + 15.635$

$50 = 32.27x + 15.635$

$x = (50 – 15.635) / 32.27$

$x = 1.064920979$

$LC_{50} = 10^{1.064920979}$

$LC_{50} = 11.61$ mg/mL

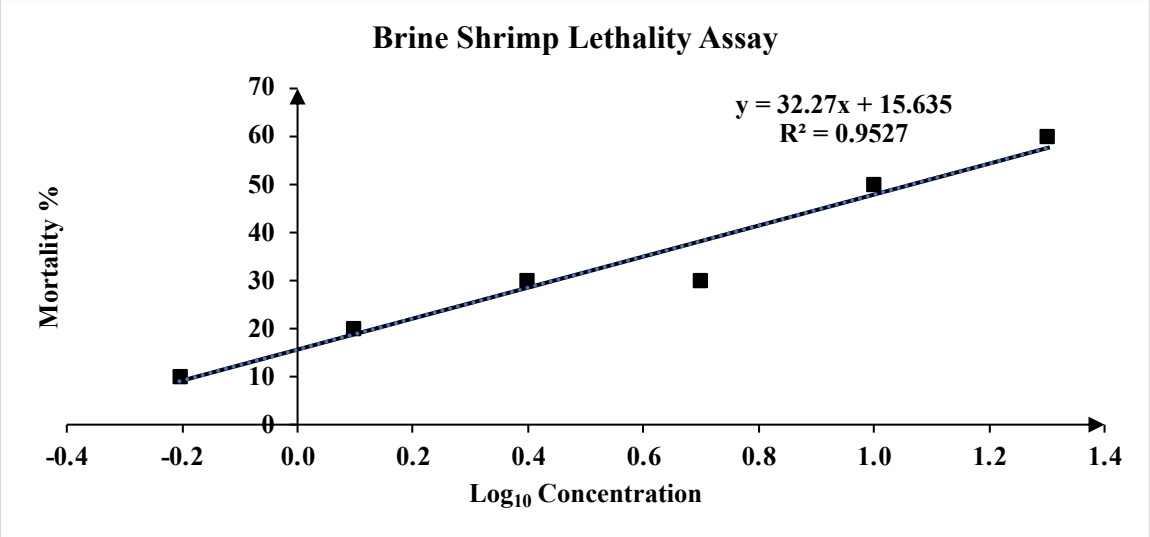

Fig. 2. Brine shrimp lethality assay of *T. catappa* L. extract.

LC_{50} value obtained in the tested extract was more than 1 mg/mL (11.61 mg/mL), indicating the extract is biologically safe and non-toxic for application in the food industry. This is by Meyer's toxicity index where he explains crude plant extract value of LC_{50} lesser than 1 mg/mL is toxic but if it is above 1 mg/mL, then it is safe for human consumption. Meyer's toxicity index adheres to date on plant studies in many areas, including food studies and the pharmaceutical field (Ohikhena *et al*., 2016; Ebadollahi-Natanzi, 2018; Nadeem *et al*., 2022).

Though limited study has been carried out so far, on LC_{50} toxicity of *T. catappa* L. leaf extract, the result obtained in this assay can be supported by a study, performed by Dash *et al*. (2022) where three solvents (aqueous, methanol, and acetone) were used separately for extraction of *T. catappa* L. leaf and found toxicity level determined in all three extractions to kill brine shrimps were much greater than 1 mg/mL, indicating the extract is non-toxic. However, the application of the extract is not advised to be greater than its LC_{50} value of 11.61 mg/mL in the food industry. As aforementioned, *T. catappa* L. leaf extract contains lots of bioactive components such as tannins, saponins, terpenoids, flavonoids, flavones, and so on. Therefore, the high content of bioactive components contributes to a possible reason for the toxicity limitation of this extract at an LC_{50} value of 11.61 mg/mL (Sarwar *et al*., 2016).

Potassium dichromate was used as positive control because it is a highly toxic compound that has been extensively studied for its toxicity to various organisms, including aquatic species. Its toxicity is well-documented, making it a reliable reference substance for evaluating the toxicity of other compounds. Apart from that, using a known toxic substance like potassium dichromate provides a standardized basis for comparison as many researchers have used this compound as a positive control in brine shrimp lethality assay (Ramli *et al*., 2020; Juwitaningsih *et al*., 2021). The use of solvent control is a fundamental practice in toxicity testing to account for any potential effects of the solvent itself on the test organisms. In the current study, 10% DMSO served as the solvent control, allowing us to discern whether any observed effects on brine shrimp lethality are attributed to the brine shrimps or the solvent. Similarly, in a study carried out by Ngassapa *et al*. (2022), DMSO was used as a negative control based on the concentration used to dissolve the extract.

CONCLUSION

Ten phytochemical compounds have been identified in *T. catappa* L. extract using GC-MS and were all found to have several biological activities reported, demonstrating the potential of this extract to be used as a lead ingredient in developing antibacterial agents in future studies. In the future, fractionation of the extract could be done to isolate the compounds and explore the synergetic effect on desired biological activities as some compounds might work more effectively when combined, enhancing the overall bioactivity. Successful synergetic investigations can lead to the development of novel formulations and applications in many sectors. Many plants across the world have been claimed to have antibacterial activities but their cytotoxicity evaluation is of utmost importance to ensure the extract's application in food substances because they might as well be harmful to consumers upon consumption at a high level. The cytotoxicity test using the brine shrimp lethality assay revealed that *T. catappa* L. extract was not cytotoxic, which is relevant as it is commonly used as an herbal plant in folk medicines, where its fallen leaves were consumed as tea. Therefore, the extract is biologically safe and non-toxic. In the future, *in vivo* studies can be conducted on animals such as rats to assess toxicity in a whole-organism context as *in vivo* studies provide a better understanding of systemic effects and potential interactions.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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