

# Comparative Cytotoxicity of Roasted Coffea Arabica And Leucaena Leucocephala Seed Extract Using Brine Shrimp Lethality Assay

DANTE A. BARBERO

Master of Arts Teaching Biology  
Saint Mary's University

**Abstract:** Coffee comprises compounds that contribute to the flavor and bioactivity of the brew. The complex reaction modifies the coffee's chemical composition during roasting at high temperatures, with some beneficial compounds and some created. The *Leucaena leucocephala* is locally termed as "Ipil-ipil tree.". Findings have revealed that it has antidiabetic and antioxidant properties. This study aims to analyze, determine and compare the level of cytotoxicity of *Coffea arabica* and *Leucaena leucocephala* using the brine shrimp lethality assay and validate the nutritional value, food edibility, and medical benefits of *Leucaena leucocephala* to be considered as one of the substitutes for coffee consumption. The general findings revealed that *C. Arabica* has (LC<sub>50</sub>) 10584.75 and *L. leucocephala* has (LC<sub>50</sub>) 1100.286, which indicates that both compounds have no toxicity. These findings are based on Meyers and Clarkson toxicity indexes. This study recommends that *L. leucocephala* seeds could be eaten and substituted for coffee, but consumption should be in moderation.

## INTRODUCTION

The *Coffea arabica* belongs to the Rubiaceae family genus *Coffea*. Although more than 80 species have been identified worldwide (Adriana, 2012) Coffee comprises compounds that contribute to the flavor and bioactivity of the brew. The complex reaction modifies the coffee's chemical composition during roasting at high temperatures, with some beneficial compounds and some created. A small amount of harmful substance is also produced during roasting; however, the beneficial compounds appear to predominate (A primeron, 2012) The *Leucaena Leucocephala*, locally termed as "Ipil-ipil tree," which belongs to Leguminosae, is a giant, dense and quick growing tree used for forage (National Research Council, 1977) When ripe, the fruit is an oval or linear pod that is strapshaped, 12 to 18 centimeters long, 1.4 to 2 centimeters wide, papery, green that becomes brown and splits open along two edges, with multiple fruits forming from each flower head. Each pod has 15 to 25 elliptic, compressed, shiny brown seeds that are 5 to 8 millimeters long and 3 to 5 millimeters wide. (Ipil-ipil, n.d.) Moreover, it produced a superior digestibility coefficient and nutrient utilization values (Sotolu & Faturi, 2008) Generally, the tree normalizes the soil acidity, salinity, and soil erosion (Shukla et al., 2002). The analyzed nutrient constituents showed that *Leucaena* seeds are a possible source of protein and energy. (Ahmed & Abdelati, 2009) Antidiabetic effects were found in the inactive fraction of *L. leucocephala* seeds in alloxan-induced rats treated, with bioactive molecules showing glycoside metabolites with galactose saccharides clusters and other sugars. (Syamsudin & Simanjuntak, 2010) In addition, The impact of an ethyl acetate fraction from *L. leucocephala* extract derived from macerated dry and powder seeds on blood sugar levels in alloxan provides more efficient hyperglycaemic Wistar rats was investigated. The EA fraction reduced glucose levels (p0.05) more than the negative control. (Tri Bowo et al., 2012) A study of seed oil revealed anti-diabetic and antioxidant properties. The amount of serum insulin increased in rats treated. The total phenolic compounds were credited with the antioxidant activity. (Chowtivannakul et al., 2016) Cytotoxicity is the state of being lethal to the cell; cells exposed to cytotoxic

compounds can respond somehow. The cell may undergo necrosis in which they lose membrane integrity and die rapidly due to cell lysis. They can stop growing and dividing and activate a generic program of controlled cell death termed apoptosis. (Hamidi et al, 2014) The Brine shrimp is a rapid and reliable, inexpensive bioassay to active plant extract. The procedures allow the determination of LC<sub>50</sub> in µg/mL of active constituents in the brine medium. This Bioassay has been used in the analysis and others of the natural product, pesticide residues, and other chemical pollutants in the marine environment. The result of this study will be an eye-opener for the readers to consider the maximum benefits of *Leucaena leucocephala* for its nutritional value, food edibility, and pharmacological benefits as a substitute for coffee consumption. This study aims to analyze, determine and compare the level of cytotoxicity of *Coffea arabica* and *Leucaena leucocephala* and validate the nutritional value, food, and medical benefits of *Leucaena leucocephala* to be considered as one of the substitutes for coffee consumption.

## METHODOLOGY

### 1.1 Preparation of materials and samples

The plant seed resources, *L. leucocephala* and *C. arabica* were collected at Pinaripad Norte, Aglipay Quirino 3403 Philippines. The collected seeds of plant samples were dried for seven days under the sun and then subjected to a standard roasting procedure. Then the roasted seeds of the plant sample were grounded using a miller.

### 1.2 Preparation of Brine Solution

The 3.6 grams of rock salt was dissolved into 100 mL of distilled water, and this will serve as artificial seawater for *Artemia salina*.

### 1.3 Hatching the Shrimp Eggs

A clean-clear rectangular container was used for brine shrimp hatching. It was divided into two unequal compartments with a paper with a small hole dividing into two chambers; a minute brown brine shrimp eggs were sprinkled into the larger compartment. The large room was

covered to keep away from sunlight, the smaller container left open for illumination. After 48 hours, the hatched brownish-orange nauplii were present in smaller illuminated parts. The lighting has little effect on hatching survival than nauplii exposure to natural light or no light. The hatching potential in a completely dark setting is 90% (Quazi et al., 2017)

#### 1.4 Bioassay

The nauplii were obtained from the container using a dropper and counted microscopically using a magnifying glass held against a well-lighted background. The nauplii are not fed during the time of the experiment. The nauplii may have died due to the plant extract's toxicity or starvation. A control sample of only nauplii has been used to establish that the plant extract has a mortality impact.

#### 1.5 Plant Test extract preparation

The ground seed materials weighed 0.04 grams with the use of digital balance. The 0.04 ground seeds were soaked in a 10 mL brine solution for 24 hours in a clean bottle container. The concentration for each vial was derived from serial dilution. The diluted ground seed materials in brine shrimp solution were undergone into serial dilution

in vials. Each control vial contains 3 mL of the prepared concentration. Ten nauplii were transferred in each sample concentration labeled (2000ppm, 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm), respectively then the vials were kept illuminated. The mortality rate was monitored and counted using a magnifying glass after 24 hours.

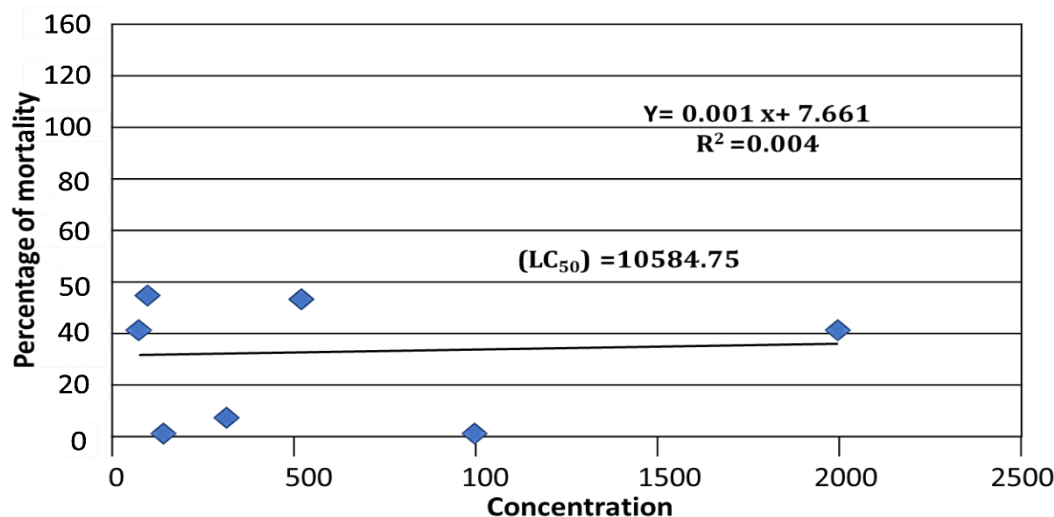
#### 1.6 Determination of Median Lethality Concentration $LC_{50}$

The mortality rate of the nauplii was determined by percentage. The toxicity of the tested concentrations was determined by comparing the lethal concentration ( $LC_{50}$ ). Probit analysis will be used to calculate the lethality concentration ( $LC_{50}$ ). The regression line created by graphing the concentration against the death percentage on a probit scale will be used to derive the ( $LC_{50}$ ) (median lethal concentration).

## RESULTS AND DISCUSSION

Studies have demonstrated that hatched nauplii are unique for early cytotoxicity testing. The majority of researchers prefers this strategy. (Carballo et al., 2002)

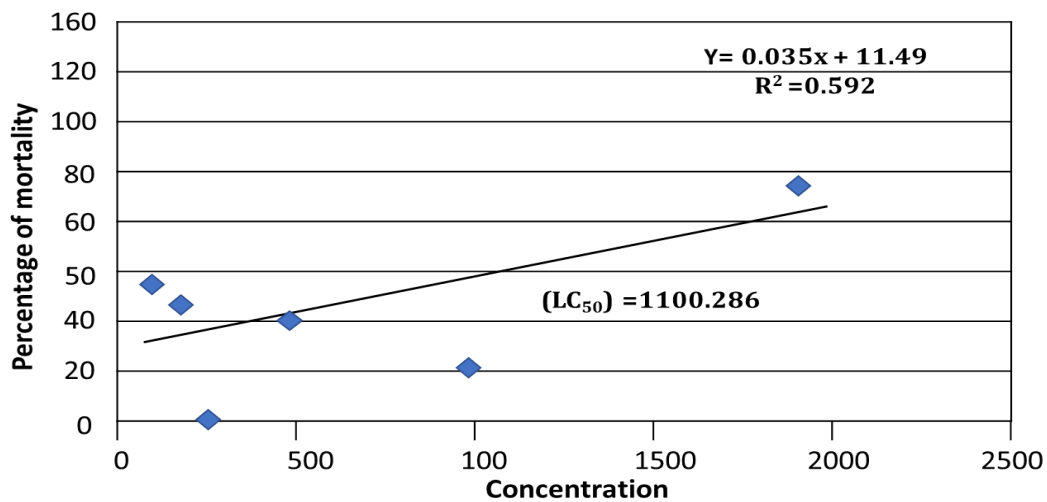
**Figure 3.1** Obtaining the logarithmic value of concentration by interpolation from the linear correlation between probit and  $\log(c)$  of *Coffea arabica*.



The preceding graph is the percent mortality of *C. arabica* in 24 hours. The acquired logarithmic value of the concentration by interpolation from the linear correlation probit and  $\log(c)$  was low. This means that the *C. arabica* has a low toxicity in the brine shrimp (*Artemia salina*) model. It was also revealed above that the lethal concentration of *C. arabica* was ( $LC_{50}$ ) 10584.75, indicating a very low toxicity level of *C. arabica*. Therefore it is not toxic. These findings are based on Meyer's toxicity index, wherein the extract with  $LC_{50}$  lower than 1000  $\mu\text{g/mL}$  is considered toxic, and the crude extracts with  $LC_{50}$  higher than 1000  $\mu\text{g/mL}$  are considered nontoxic. (Hamidi et al., 2014) This claim is also supported by Clarkson toxicity assessment with classified extract according to order: plant extract with ( $LC_{50}$ ) above 1000  $\mu\text{g/mL}$  is nontoxic, extract with ( $LC_{50}$ ) of 500 to 1000  $\mu\text{g/mL}$  is low toxic, with ( $LC_{50}$ ) of 0 to 100  $\mu\text{g/mL}$  are highly toxic (Unuofin et al., 2017). Therefore, the

*Coffea arabica* has no toxicity and is considered highly consumable. Studies have also reported that it is safe for most people when coffee is ingested in low-to-moderate levels. (Temple et al., 2017) This claim was supported by an article (Shaikh, 2020) that most adults are deemed safe to consume up to 400 mg of caffeine per day. This equates to approximately four cups of coffee (1 cup Equals 8 ounces). However, a study showed that persons who drink more than six cups of coffee per day are more likely to have poor health, earlier mortality, and a lower quality of life due to these disorders. (Excess coffee..., 2020) While the previous study has focused on the negative aspects of coffee, new research suggests that it may have medical benefits. The antioxidant or anti-inflammatory compounds found in coffee are responsible for the help. These substances have been linked to illness prevention in your body. (Shaikh, 2020)

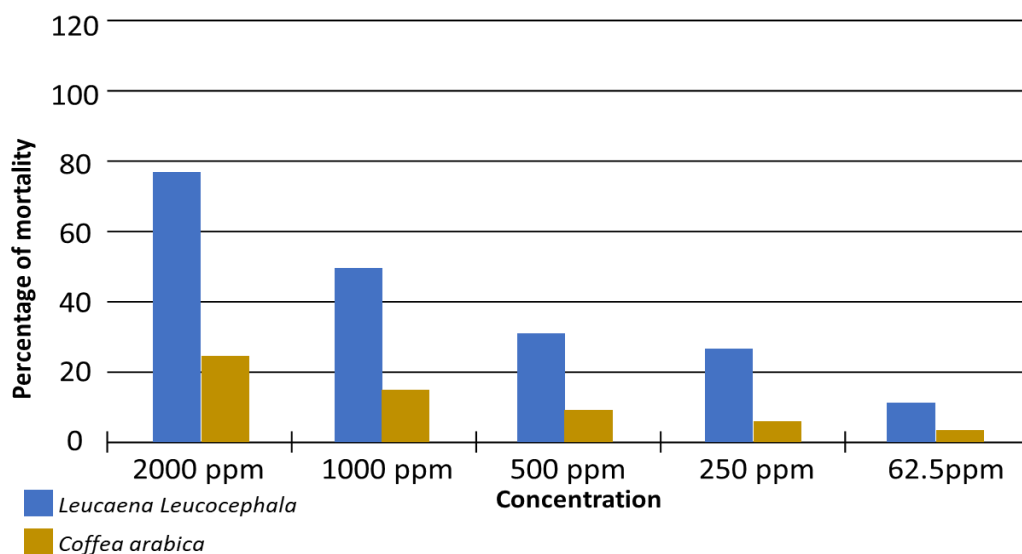
Figure 3.1 Obtaining the logarithmic concentration value by interpolation from the linear correlation between probit and log (c) of *Leucaena leucocephala*.



The preceding graph is the percent mortality of *L. leucocephala* in 24 hours. The acquired logarithmic value of the concentration by interpolation from the linear correlation probit and lof (c) was high. This means that there was a bit of toxicity of *L. leucocephala* in the brine shrimp (*Artemia salina*) model. It was also revealed that the lethal concentration was ( $LC_{50}$ ) 1100.286, indicating the toxicity level of *L. leucocephala* is moderately low. Consequently, it is not toxic. These findings were based on Meyer's toxicity index, wherein the extract with  $LC_{50}$  lower than 1000  $\mu\text{g/mL}$  is considered harmful, and the crude extracts with  $LC_{50}$  higher than 1000  $\mu\text{g/mL}$  are considered nontoxic (Hamidi et al, 2014) . This claim was also supported by Clarkson toxicity assessment with classified extract according to order: plant extract with ( $LC_{50}$ ) above 1000  $\mu\text{g/mL}$  is nontoxic, extract with ( $LC_{50}$ ) of 500 to 1000  $\mu\text{g/mL}$  is low toxic, extract with ( $LC_{50}$ ) of 0 to 100  $\mu\text{g/mL}$  are highly toxic. (Unuofin et al, 2017) Therefore, the *L. leucocephala* has no toxicity, and it is considered to be consumable. Findings revealed that the seeds and leaf have a high protein, fat, and

carbohydrate content. Furthermore, it could serve as a good source of animal feed owing to its high nutritional value and nontoxic effect (Afza et al., 2007). It is also used as human food (Perez- Gil et al., 1987). *L. leucocephala* has the potential to be used as a natural remedy against diseases (Erdingil et al., 2007). Medically it has been studied for tumor cells (Itzhaki & Abdulla, 1982). As an anti-fertility agent (Monzon et al., 1995), it has fungicidal properties (Shukla et al., 1990) Ipil-ipil seeds increase the level of Provitamin in chicken (Chou et al., 1964). Along with the highly beneficial effect of *L. leucocephala*, finding revealed that it has Mimosine, a harmful non-protein organic compound; overconsumption of *L. leucocephala* causes baldness, development retardation, cataract, goiter, decreased fertility, and mortality in non-ruminants, provides up to 14.8% of the total nitrogen content of *L. leucocephala* seeds. (Kale, 1987.) The mimosine concentration ranges between 2.2% and 10%. (Kewalramani et al., 1987)The content of mimosine in the seeds of the different cultivars of the plant varies considerably. (Ter et al., 1979).

Figure 3.3 The comparative cytotoxicity of *C. Arabica L.* and *L. leucocephala*



The above chart shows the comparative cytotoxicity of *C. Arabica* L. and *leucocephala*. This shows the mortality rate of brine shrimp (*Artemia salina*) in each concentration. We could observe that the *Leucaena leucocephala* concentration has a high mortality rate percentage compared to *Coffea arabica*, with a low mortality rate. The general findings revealed that *C. Arabica* and *L. leucocephala* have no toxicity, but there is a big difference in the concentration prescribed for consumption.

## Conclusion

The brine shrimp lethality bioassay is a rapid and easy cytotoxicity test for bioactive compounds. It is based on the capacity of test substances to kill nauplii, a simple zoological species (*Artemia salina*). A maximum concentration of plant extract contributes to significant mortality of the brine shrimp. The result revealed that *C. Arabica* L. and *L. leucocephala* have no toxicity on the brine shrimp *Artemia salina* model. It is true; *Coffea arabica* was considered a primary beverage because it has nutritional value, and *L. leucocephala* was deemed to be beneficial because it has medical and industrial purposes. Generally, *L. leucocephala* seeds could be eaten and substituted for coffee, but consumption should be in moderation.

## References

- [1]. A primer on... (2012). A primer on pef's priority commodities; industry study on coffe. Coffe production data, Bureau Of agricultural statistics.
- [2]. Adriana, F. (2012). Coffee constituent; Emerging health effects and prevention . Blackwell Publishing .
- [3]. Afza et al. (2007). Physico-chemical and toxicological studies of different parts of *Leucena leucocephala*. Pakistan Journal of pharmacologypharmaceutical Research Center PCSIR Lbaoratories Complex , 13-16.
- [4]. Ahmed, M. E., & Abdelati, K. A. (2009). Chemical Composition and Amino Acids Profile of *Leucaena leucocephala* Seeds. International Journal of Poultry Science, DOI: 10.3923/ijps.2009.966.970.
- [5]. Carballo et al. (2002). A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. . BMC Biotechnol., 17.
- [6]. Chou et al. (1964). Comparative Vitamin K activity of Dehydrated Alfallfa and *Leucaena Leucocephala* Meal . Poultry Sci. 44, 97.
- [7]. Chowtivannakul et al. (2016). Antidiabetic and antioxidant activities of seed extract from *Leucaena leucocephala* (Lam.) de Wit /. Agriculture and Natural Resources, 357-361.
- [8]. Erdingil et al. (2007). Chemical Composition and Biological activity of The essential Oil of *Leucena Leucocephala* . Pharma. Biol. 45, 324.
- [9]. Excess coffee... (2020). Excess coffee consumption a culprit for poor health. University of South Australia, Retrieved from <https://www.unisa.edu.au/Media-Centre/Releases/2020/excess-coffee-consumption-a-culprit-for-poor-health/>.
- [10]. Hamidi et al. (2014). Toxicological eveluation of the plant products Using the Brine Shrimp Model . Faculty of Pharmacy, Institute of applied Bicehemistry, 9-18.
- [11]. Hamidi et al. (2014). Toxicological evaluation of the plant products using brine shrimp (*Artemiasalina* L.) model. Macedonian Pharmacy Bulletin 60(1);, 9-18.
- [12]. Ipil-ipil. (n.d.). List of Philippine Medicinal Plants with Chinese Names, Retrieved from <http://www.stuartschange.org/Ipil-ipil>.
- [13]. Itzhaki, S., & Abdulla, P. (1982). Effects of Mimosine on RNA Synthesis in Ehrlich Ascite-Tomour Cells. BioChem Soc Tarns , 176.
- [14]. Kale. (1987.). Nutritive value of *Leucaena leucocephala* (subabul). . Doctoral thesis, University of Bombay, .
- [15]. Kewalramani et al. (1987). Proximate composition, mimosine and mineral contents of *Leucaena* sp. and hybrids. . Indian J Anim Sci , 57:1117-2.
- [16]. *Leucaena*. (2012). Fruits info.com, Retrieved from <https://www.fruitsinfo.com/leucaenafruit.php#:~:text=The%20plant%20is%20believed%20to,back%20pain%20and%20menstrual%20cramps>.
- [17]. Monzon et al. (1995). Traditional medicine in the treatment of Parasitic Diseases in the Philippines . South East Asion Journal of tropical Medicine and Public Health 26(3), 421.
- [18]. National Research Council. (1977). *Leucena: Prosmising forage and tree crops for the tropics* . Washington DC National Academi press, <https://doi.org/10.17226/21315>.
- [19]. Perez- Gil et al. (1987). Traditional and Non-traditional Food: Chemical Composition of *Leucena esculenta* and its utilization as Human food. Techno. Aliment, (Mexico City), 20, 22, 24, 26.
- [20]. Quazi et al. (2017). Brine shrimp lethality assay. Bangladesh J Pharmacol., 186-189.
- [21]. Shaikh, J. (2020). What Are the Negative Effects of Coffee? Medicine. net , Retrieved from [https://www.medicinenet.com/what\\_are\\_the\\_negative\\_effects\\_of\\_coffee/article.htm](https://www.medicinenet.com/what_are_the_negative_effects_of_coffee/article.htm).

- [22]. Shukla et al. (1990). Effects of Fungicidal Seed Dressing and Other Treatment on the Germination and growth of subadul (Leucocephal ) De wit. Indian J forest 13, 97.
- [23]. Shukla et al. (2002). Evaluation and uptake from Minjingu Phosphate rock and growth of tree species growing on an acid soil from western kenya using P-Lsotope Dilution technique. National Agricultural Research laboratories, 67.
- [24]. Sotolu, A., & Faturi, E. (2008). Digestibility and nutritive values of different Porcessed Leucaena leucocephala seed meals in the Diet of African Cat fish . Middle East Journal Of Scientific Research , 190.
- [25]. Syamsudin, R. S., & Simanjuntak, P. (2010). Antidiabetic Activity of Active Fractions of Leucaena Leucocephala (Imk) Dewit Seeds in Experiment Model. European Journal of Scientific Research,, pp.384-391.
- [26]. Temple et al. (2017). The Safety of Ingested Caffeine: A Comprehensive Review. Pub Med.gov, 10.3389/fpsy.2017.00080.
- [27]. Ter et al. (1979). A review on the nutritive value and toxic aspects of Leucaena leucocephala. Trop Anim Prod, 4:113-26.
- [28]. Tri Bowo et al. (2012). Antidiabetic Drug Ethyl Acetate Fraction of Leucaena leucocephala Seed Extract in Wistar Aloxan Induced. International Conference: Research and App.
- [29]. Unuofin et al. (2017). Toxicity Assessment Of Kedrostis Africana Cogn: A Medicinal Plant Used In The Management Of Obesity In South Africa Using Brine Shrimp (Artemia Salina Linn.) Assay. Internation Journal of Pharmaceutical Sciences and Research.