## Research Article

# Antioxidant Capability and Inhibitory Effect of Leaf Extracts of *Albizia lebbeck (L.)* Benth. Against Formation of Mutagen and Mutagenesis

**Wannagorn Khunnasarn[1], Chadamas Promkum[2], Rianthong Phumsuay[3], Sarunya Kitdumrongthum[2], Chaniphun Butryee[2], Chawanphat Muangnoi[3], Monruedee Sukprasansap[2*]**

[1]*Graduate student in Master of Science Program in Toxicology and Nutrition for Food Safety, Institute of Nutrition, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand.*

[2]*Food Toxicology Unit, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand.*

[3]*Cell and Animal Model Unit, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand.*

**ABSTRACT**

*Albizia lebbeck* (L.) Benth. contains several pharmacological properties and has potential health benefits. In Thailand, this plant is called Ta-kuk, the young leaves of which are eaten by local people and commonly prepared by boiling. Since data are scarce on the antiformation of mutagens and antimutagenic activities of these young edible leaves. This study investigated the effect of different extracts obtained from edible leaf of *A. lebbeck* on the reducing the formation of mutagens, and antimutagenic activity by the Ames test using *Salmonella typhimurium* strains TA98 and TA 100 models without metabolic enzyme activation. Nitroaminopyrene (0.037 µg/plate) was used as a standard mutagen in our Ames test model. *A. lebbeck* leaves were extracted by ethanol, mix solvents, hexane, and water. Results showed that extracts exhibited strong antioxidant activities and were high in total flavonoid and total phenolic contents. In the Ames test, results showed that each extract had no toxic or mutagenic effects at various concentrations (375 - 6000 µg/plate) for both strains. These extracts could inhibit the formation of mutagens at moderate to strong activity, while *A. lebbeck* leaf aqueous water extract showed none to moderate capability. Moreover, all extracts showed a strong potential antimutagenicity for both TA 98 and TA 100, with criteria percentage inhibition at over 60%. Overall, these findings demonstrate that edible young leaves of *A. lebbeck* have antioxidant properties that could prevent the formation of mutagens and antimutagenicity against nitroaminopyrene direct-acting mutagenesis.

**Keywords:** *Albizia lebbeck* (L.) Benth; Antioxidant; Ames test; Antimutagenicity; Formation of mutagen; Nitroaminopyrene

Received: 21 May 2024 Accepted: 30 May 2024 Available online: 3 June 2024

* Correspondence: monruedee.suk@mahidol.edu

## บทความวิจัย

# ความสามารถในการต้านอนุมูลอิสระ และฤทธิ์การยับยั้งของสารสกัดจากใบตะคึกต่อการเกิดสารก่อกลายพันธุ์และการกลายพันธุ์

**วรรณกร คุณสาร[1] ชฎามาศ พรหมคำ[2] เหรียญทอง พุ่มสวย[3]**

**ศรัณยา กิจดำรงธรรม[2] ชนิพรรณ บุตรยี่[2] ชวัลพัชร เมืองน้อย[3]**

**มลฤดี สุขประสารทรัพย์[2*]**

*[1]วิทยาศาสตรมหาบัณฑิต สาขาวิชาพิษวิทยาและโภชนาการเพื่ออาหารปลอดภัย*

*สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย*

*[2]หน่วยพิษวิทยาทางอาหาร สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย*

*[3]หน่วยเซลล์และสัตว์ทดลองต้นแบบ สถาบันโภชนาการ มหาวิทยาลัยมหิดล นครปฐม ประเทศไทย*

**บทคัดย่อ**

ตะคึก (*Albizia lebbeck* (L.) Benth.) เป็นพืชที่มีคุณสมบัติทางเภสัชวิทยาและประโยชน์ต่อสุขภาพหลายประการ ในประเทศไทยคนท้องถิ่นนิยมนำใบอ่อนของต้นตะคึกมาปรุงสุกเป็นอาหารเพื่อรับประทาน เช่น วิธีต้ม เป็นต้น อย่างไรก็ตาม ยังไม่มีการศึกษาข้อมูลฤทธิ์ของใบอ่อนตะคึกส่วนที่รับประทานได้ต่อฤทธิ์การต้านการเกิดสารก่อกลายพันธุ์และต้านการก่อกลายพันธุ์ ดังนั้น การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อทดสอบฤทธิ์ของสารสกัดจากใบตะคึกส่วนที่รับประทานได้ที่สกัดด้วยตัวทำละลายชนิดต่าง ๆ ต่อการลดการเกิดสารก่อกลายพันธุ์และการต้านการก่อกลายพันธุ์ด้วยการทดสอบเอมส์โดยใช้ *Salmonella typhimurium* สายพันธุ์ TA98 และ TA100 ระบบที่ไม่มีระบบเอนไซม์กระตุ้น ไนโตรอะมิโนไพรีน (0.037 ไมโครกรัม/เพลท) ถูกใช้เป็นสารก่อกลายพันธุ์มาตรฐานในการทดสอบโมเดลเอมส์ ในการศึกษาครั้งนี้ใบตะคึกส่วนที่รับประทานได้จะถูกสกัดด้วยเอทานอล ตัวทำละลายผสม เฮกเซน และน้ำ สารสกัดเหล่านี้มีฤทธิ์ต้านอนุมูลอิสระสูงและมีฟลาโวนอยด์รวมและฟีนอลิกรวมสูง ในการทดสอบเอมส์ผลการทดลอง พบว่า สารสกัดแต่ละชนิดไม่มีพิษและไม่มีฤทธิ์การกลายพันธุ์ที่ความเข้มข้นต่าง ๆ (375 - 6000 ไมโครกรัม/เพลท) ทั้งสายพันธุ์ TA98 และ TA100 สารสกัดทั้งหมดสามารถยับยั้งการเกิดสารก่อกลายพันธุ์ได้ในระดับปานกลางถึงมาก ในขณะที่สารสกัดน้ำของใบตะคึกมีความสามารถยับยั้งถึงปานกลางเท่านั้น ยิ่งไปกว่านั้นสารสกัดเหล่านี้แสดงให้เห็นถึงศักยภาพในการออกฤทธิ์ต้านการกลายพันธุ์ในระดับสูงทั้งในสายพันธุ์ TA 98 และ TA 100 โดยเปอร์เซ็นต์การยับยั้งเผยให้เห็นมากกว่า 60% ในตัวอย่างทั้งหมด โดยรวมแล้วการค้นพบนี้แสดงให้เห็นว่าใบอ่อนของตะคึกในส่วนที่รับประทานได้นี้มีคุณสมบัติต้านอนุมูลอิสระที่สามารถป้องกันการเกิดสารก่อกลายพันธุ์และมีฤทธิ์ต้านการก่อกลายพันธุ์ต่อการก่อกลายพันธุ์ที่ออกฤทธิ์โดยตรงของไนโตรอะมิโนไพรีน

**คำสำคัญ:** ตะคึก ฤทธิ์ต้านอนุมูลอิสระ การทดสอบเอมส์ การต้านการกลายพันธุ์ การเกิดสารก่อกลายพันธุ์ ไนโตรอะมิโนไพรีน

* Correspondence: monruedee.suk@mahidol.edu

## INTRODUCTION

A mutation is a change in a small section of a genome's nucleotide sequence in which one nucleotide is frequently replaced by another in point mutations. Other changes involve the insertion or deletion of one or more nucleotides. Errors in DNA replication or the harmful effects of mutagens, such as chemicals and radiation that react with DNA and alter the architecture of individual nucleotides, are the two main causes of mutation[1]. Unfortunately, mutagenic and carcinogenic mediators are all-pervading in our environment. DNA damage ensues throughout an organism's life cycle due to exogenous and endogenous factors[2-4]. The DNA damage response can affect apoptosis and may cause genomic instability, mutation, unfettered cell growth, and an increased risk of cancer[5-7]. Cancer is one of the most harmful groups of human pathologies, exhibiting a wide range of hallmark clinical structures and causing a significant amount of mortality each year and globally. Cancer accounted for approximately 10 million deaths in 2020, or roughly one in every six deaths[8-9]. Cancer is a metabolic and signaling disorder that results in unchecked cell division and survival. Of the different forms of cancer, gastric cancer is among the top 3 most prevalent cancers in 19 countries and was the most commonly diagnosed cancer in four of them (Bhutan, Kyrgyzstan, Cape Verde, and Tajikistan) according to a projection of 1.1 million cases (720,000 men and 370,000 women) in 2020. In total, 60% of all instances of stomach cancer were found in Eastern Asia, with 43.9% of those cases in China alone[10]. For Thailand specifically, the most recent WHO data indicated that there were 3,221 stomach cancer deaths or 2.6% of all deaths in 2020. The annual incidence adjusted for age is 3.50 per 100,000 people[8]. According to Thailand's National Cancer Institute, stomach cancer is the eighth most prevalent cancer in men (2.6% of cases) and the ninth most common cancer in women (1.6% of cases)[11]. Due to its tragic prognosis, stomach (gastric) cancer continues to be a serious health issue. Consequently, preventive approaches to combat mutation and cancer are critical in order to control and prevent the risk of mutagenesis and cancer disorders, including stomach cancer. Towards this end, plants and dietary natural products may play a role in the prevention and treatment of cancer and aid against DNA damaging processes[12-13]. Natural and botanical products contain many phytochemicals that benefit human health, especially substances with antimutagenic and anticarcinogenic properties[14-16]. It has been established that cereals, fruits, and vegetables are preventative against the onset of many chronic illnesses. Numerous phytochemicals derived from plants have been found to be cytotoxic to tumors, such as tannins, flavones, triterpenoids, steroids, saponins, and alkaloids[13]. Additionally, many organic substances have been shown to induce cell cycle arrest, stimulate apoptosis, obstruct the development of cancer cells, and prevent angiogenesis[17]. *Albizia lebbeck* (L.) Benth. is a plant used in traditional medicine. It is found in tropical countries including Brazil, Peru, Indian, Myanmar, and Thailand. *A. lebbeck* (L.) Benth. is called Ta-kuk or golden jamjuree in Thai. Several phytochemicals are present in this plant, including alkaloids, anthraquinones, essential oils, flavonoids, glycosides, phenolics, phytosterols, saponins, steroids, and triterpenoids[18-19]. Over time, native edible plants

like *A. lebbeck* have become crucial parts of food systems connected to food security and are significant sources of nutrients and possible health benefits. In Thailand, the young leaves of this plant are eaten by local people and commonly prepared by boiling. These leaves contain high levels of vitamin C, total contents of phenolic compounds, antioxidant activity, flavonoids, carotenoids, and dietary fiber[20-22]. *A. lebbeck* has several pharmacological attributes including having antibacterial, anti-inflammatory, antioxidant, anticancer, anti-ulcer, anti-diarrheal, and immunomodulatory properties[18, 21-25]. Stem bark extract of *A. lebbeck* has been shown to have a strong antimutagenic effect, lowering the rate of base substitution mutations for *Salmonella typhimurium* strain TA100 by 94.66%. It is possible that stem bark aqueous extracts from this plant can shield cells from naturally occurring gene alterations[26]. However, evaluations of the mutagenic activity of edible leaf extracts from *A. lebbeck* (L.) Benth. are limited. Hence, the aim of this present study was to investigate the effects of *A. lebbeck* (L.) Benth. derived from different leaf extracts against nitroaminopyrene direct-acting mutagenesis by the Ames test using *S. typhimurium* model and their antioxidant capability.

## Materials and methods

### Sample preparation and extraction

Young leaves of *A. lebbeck* or Ta-kuk were collected from the conservation area of the Electricity Generating Authority of Thailand, Srinakarind Dam, Kanchanaburi province, as part of the Plant Genetic Conservation Project initiated by Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG). The scientific name of this plant was identified and authorized by Asst. Prof. Dr. Thaya Jenjittikul, Department of Plant Science, Faculty of Science, Mahidol University, Thailand. A voucher specimen was deposited at Suan Luang Rama IX Herbarium, Bangkok, Thailand, under the designation of No. 9429. The young leaf sample was prepared following the procedure of Phoraksa and colleagues (2023)[20]. Powdered samples were packed in aluminum foil bags under vacuum and stored at -20 ºC until use. Samples were extracted with three different polarities of organic solvents, including hexane, mix solvents [hexane: acetone: ethanol at a ratio of 2:1:1 (v/v/v)], and 95% ethanol at a solvent ratio of 1:15 (w/v)[20] by the maceration method at room temperature for 24 h in a shaker machine[26]. Thereafter, the mixture for each extract was centrifuged and filtered through a filter paper, while each sample's sediment was further extracted twice with four different solvents. The supernatants were collected and then evaporated under vacuum at 35-40 °C until dry. Subsequently, each crude extract was blanked with nitrogen gas until dry and stored at -20 °C in darkness. Water extraction from the leaves was performed using the maceration method according to procedures modified from previous studies[27-28]. We used the ratio of solvent as mentioned above. Thereafter, the solution mixture was centrifuged and filtered through filter paper. Subsequently, this aqueous water extract was stored at -20 °C in darkness.

### Determination of antioxidant properties of sample

#### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was conducted with some procedural adaptations as described by Kongkatitham et al. (2018)[29] and Sukprasansap et al. (2019)[30]. Briefly, samples were introduced

to individual wells of a 96-well plate at a concentration of 50 g/ml (22 µl), accompanied by the addition of 200 µl of 150 M DPPH in methanol. The plate was covered and placed in a light-free environment at an ambient temperature for 30 minutes. Subsequently, absorbance was measured at 520 nm by a microplate reader. Results were presented as mmol Trolox equivalent (TE)/g sample and the percentage of radical scavenging activity was determined by employing the subsequent formula: % radical scavenging activity = [(absorbance of blank - absorbance of sample) / absorbance of blank] × 100.

**Ferric reducing antioxidant power (FRAP) assay**

The FRAP reagent was prepared by combining 25 ml of acetate buffer (30 mM, pH 3.6), 2.5 ml of TPTZ solution (10 mM), and 2.5 ml of ferric chloride solution (20 mM). Subsequently, 150 µl of FRAP reagent (consisting of ferric chloride and TPTZ in an acetate buffer at pH 3.6) was mixed with 20 µl of the extract sample in a 96-well plate. The mixture was maintained in darkness for 8 min. Thereafter, the absorbance of both the sample and Trolox standard was measured at a wavelength of 600 nm using the microplate reader. Data were presented as mmol TE/g sample[3].

**Oxygen radical absorbance capacity (ORAC) assay**

This assay measured the ability to resist the production of free radicals at 37 °C under a pH 7.4 condition using peroxyl radicals triggered by 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) by measuring fluorescence reduction[31-32]. It utilized a fluorescein wavelength of 485 nm for excitation and 540 nm for emission[5] and reported the quantity of antioxidant substances (µmol TE/g) in the sample.

**Nitric oxide (NO) scavenging assay**

This method was used for assessing a solution's ability to remove nitric oxide radicals, according to the procedure of Aktas[33]. Sodium nitroprusside was dissolved in pH 7.2 buffer to generate nitric oxide, which reacts with oxygen to form nitrite, detectable using the Griess reagent. A 20 µl volume of the extract was added to a 96-well plate, followed by 40 µl of 10 mM sodium nitroprusside solution (dissolved in phosphate buffer saline), followed by incubating the mixture under fluorescent light at room temperature for 120 minutes. Subsequently, 100 µl of Griess reagent (containing 1% sulphanilamide, 0.1% N-1-napthlenediamine hydrochloride, and 2.5% phosphoric acid in a 1:1:1 ratio) was added to each well, mixed, and incubated for 10 minutes before measuring absorbance at 570 nm. Ascorbic acid served as a standard control. The scavenging effect was determined using the formula: Scavenging effect (%) = [1 - (A sample - A sample blank) / A control] × 100, where A sample and A sample blank represent the absorbance values of the extract with and without Griess, respectively, and A control is the absorbance of Griess without the extract.

**Total flavonoid content**

Flavonoid content was determined by the aluminium trichloride method using quercetin as the reference compound. An extract of 25 µl was added to 75 µl of 90% ethanol in a 96-well plate, followed by the addition of 5 µl of aluminum trichloride (10%). After mixing, 5 µl of potassium acetate was added. The final volume of the solution was adjusted to 250 µl with distilled

water. After 30 minutes of incubation, the mixture turned pink and absorbance was measured at 415 nm using a microplate reader. Total flavonoid content was expressed as mg quercetin equivalents (QE)/100 g of sample[34-35].

**Total phenolic content**

This method was based on the procedure of Sukprasansap[26, 30]. Briefly, the sample extract (10 µl) was transferred into a 96-well microplate containing 160 µl of distilled water, followed by the addition of 10 µl of Folin-Ciocalteu reagent and 20 µl of a saturated sodium carbonate solution. After incubating for 30 minutes, the mixture sample was detected by the microplate reader at 750 nm. The quantification of the total phenolic content was expressed as mg of gallic acid equivalent (GAE)/100 g of sample.

**Ames test**

**Strains of *Salmonella typhimurium***

In this study, the *S. typhimurium* strains TA98 (with a mutation in the hisD3052 allele) and TA100 (with a mutation in the hisG46 allele) were employed, along with the presence of the R-plasmid (pKM101) and mutations in rfa and uvrB. They demonstrated the ability to identify frameshift mutation (TA98) as well as base-pair substitution (TA100). Both strains were graciously obtained from the Medicinal Plant Research Institute, Department of Medical Sciences, Thailand.

**Preparation of standard direct mutagen**

1-Aminopyrene interacted with sodium nitrite to provide a direct-acting mutagen (AP-nitrite model). A volume of 10 µl (if tested with TA98) or 40 µl (if tested with TA100) of 1-aminopyrene (0.0375 mg/ml) was added into a tube, followed by 710-740 µl of 0.2 N hydrochloric acid (sufficient to acidify the reaction mixture to pH 3.0-3.4) and 250 µl of 2M sodium nitrite with thorough mixing. The final volume was adjusted to 1,000 µl with appropriate solvent. Each reaction tube was incubated at 37°C in a shaking water bath for 4 h, and the reaction was stopped by placing the tube in an ice bath for 1 min. In order to decompose the residual nitrite, 250 µl of 2M ammonium sulfamate was added and allowed to stand for 10 min in an ice bath. This final mixture or AP-product was referred to as the nitrosated product[36-39].

**Mutagenicity test**

The pre-incubation assay of the Ames test was conducted without metabolic activation, following the procedures outlined by Maron and Ames (1983)[40] and the Organization for Economic Co-operation and Development (OECD) under guideline No. 471. Firstly, each sample extract was examined for its toxicity and mutagenicity. The strains *S. typhimurium* TA98 and TA100 were grown in nutrient broth with incubation at 37 °C for 16 h, 90 rpm prior to their utilization in the subsequent experiment. A volume of 100 µl of the sample was combined with 500 µl of NaPO4-KCl buffer and 100 µl of *S. typhimurium* (TA 98 or TA100). Finally, the mixture solution was placed in a shaking water bath for 20 min at 37 °C, 120 rpm. After incubation, 2 ml of liquid top agar at 45 °C, comprising 0.6% agar, 0.5% sodium chloride, and a mixture of 0.5 mM L-histidine and 0.5 mM biotin, were transferred into individual test tubes. Each test tube was gently mixed and subsequently transferred onto a culture agar plate. After the top agar layer had hardened, the

plate was rotated to achieve uniform colony distribution and incubated for 48 h at 37 °C. The revertant colonies were observed on the test plates and evaluated in comparison to those on the negative control plates. Revertant colonies in each plate were counted and determined using a colony counter.

**Antiformation of mutagen model assay**

Non-toxic samples at different concentrations were added to the AP-nitrite model during 4 h incubation to investigate their potential to inhibit mutagen formation during incubation. An aliquot (200 µl) of each extracted sample was added at the beginning to the reaction mixture of the AP-nitrite model as described above. The final volume of the mixture was 1,450 µl. Thereafter, 200 µl of DMSO or distilled water was added in place of the sample to serve as the negative control. The mixture solution (100 µl) was added with 500 µl $NaPO_4$-KCl buffer (pH 7.4) and then treated with bacterial tester strains (100 µl TA98 or TA100), followed by incubation for 20 min at 37 °C, 120 rpm in a shaking water bath. The 2 ml molten top layer of agar (containing 0.6% agar, 0.5% NaCl, and 0.5 mM L-histidine/0.5 mM Biotin) was added, mixed well, and poured onto the minimal agar plate. After the top agar had hardened, the plate was rotated to achieve uniform colony distribution and incubated for 48 hours at 37 °C. Revertant colonies on the test plates were compared to those on the negative control and positive control plates. The number of revertant colonies in each plate was determined using a colony counter [26, 30, 37].

**Antimutagenicity model assay**

Non-toxic samples at different concentrations were examined to determine their antimutagenicity activity. The *S. typhimurium* strains TA98 and TA100 were grown in nutrient broth and incubated at 37 °C for 16 h, 90 rpm prior to their utilization in a further step. The nitrosated products were prepared according to the preparation of standard direct mutagen as previously described. A volume of 100 µl of nitrosated product was combined with 500 µl of $NaPO_4$-KCl buffer at pH 7.4, along with 100 µl of each sample extract. Subsequently, the mixture was exposed to bacterial tester strains 100 µl of TA98 or TA100 and then incubated 20 min at 37 °C, 120 rpm in the water bath shaker. The 2 ml molten top agar (containing 0.6% agar, 0.5% NaCl, and 0.5 mM L-histidine/0.5 mM Biotin) was added, mixed well, and poured onto the minimal agar plate. Once the top agar had solidified, the plate was rotated to achieve uniform colony distribution and incubated for 48 h at 37 °C. Revertant colonies on the test plates were evaluated and compared to those on the negative control and positive control plates. The number of revertant colonies on each plate was determined using a colony counter.

**Data evaluation and statistical analysis**

All results were presented as the mean ± standard deviation (SD) in at least three independent experiments. The experiments were carried out with three replicate plates for each concentration tested. Mutagenicity data were considered the criteria for assessing positive mutagenicity in the Ames test followed by the revertant colony numbers from at least two concentrations of the sample, which must be

higher than the spontaneous revertants with a concentration-response relationship, and at least one concentration with an increase in the revertant colonies is twice that of the spontaneous mutation[40-41]. The percent inhibition of samples towards the mutagens was calculated as follows: % inhibition = (A-B)/(A-C) x100, where A is a number of histidine revertants per plate induced by nitrite treated 1-aminopyrine. B is a number of histidine revertants per plate induced by nitrite treated 1-aminopyrine in the presence of the sample, and C is a number of spontaneous histidine revertants per plate (with DMSO in place of the sample). The antimutagenic potential of the sample was interpreted or considered to be strong when the inhibition was higher than 60%, moderate at 41-60%, weak at 21-40% and none when the inhibition was less than 21%, respectively[30, 39, 42]. For statistical analysis, the SPSS statistics version 29 program was used to analyze all data. One-way analysis of variance (ANOVA) was carried out in order to detect significant difference among each sample extract group in determining antioxidant properties, anti-formation of mutagen, and antimutagenicity followed by Duncan's post-hoc test. The level of significance was accepted at $p < 0.05$.

## Results

### Antioxidant activities, total flavonoid, and phenolic contents of *A. lebbeck* leaf extracts

The biological activities of the sample extracts were elucidated entailing radical scavenging activities, reducing power potential, and total flavonoid and phenolic contents, by the method described previously. As presented in **Table 1**, the results demonstrated that *A. lebbeck* leaf ethanol extract, mix solvents extract, hexane extract, and aqueous water extract had high scavenging activity for radicals for both DPPH and ORAC assays as well as NO scavenging and were strong in reducing oxidant power as measured by the FRAP assay. Furthermore, all extracts had high amounts of total flavonoids and total phenolics as shown in **Table 2**.

### Mutagenicity of *A. lebbeck* leaf extracts

Leaf extracts from *A. lebbeck* were evaluated for mutagenic activity at different concentrations (375, 750, 1500, 3000, and 6000 µg/plate) towards *S. typhimurium* TA98 and TA100 using the pre-incubation method of Ames/*Salmonella* assays without metabolic activation. Firstly, to examine the toxicity of samples, the highest concentration of each sample extract was assessed by a spot test, in which all extracts did not appear in the clear zone (data not shown). Subsequently, the mutagenicity testing of samples was investigated. The tested plates were observed the background lawn under a light microscope before counting the colonies. Results showed that all samples at the highest concentration (6000 µg/plate) exhibited no anomalies in the background lawn for both strains TA98 and TA100 (data not shown). Data of mutagenicity of *A. lebbeck* leaf extracts are shown in **Table 3** and **Figure 1**. All A. *lebbeck* leaf extracts were not toxic and mutagenic in both strains TA98 and TA100, according to the criteria for assessing the positive mutagenicity in the Ames test as mentioned above in the section on data evaluation.

**Table 1.** Antioxidant activities of *A. lebbeck* leaf extracts

| Sample | DPPH (%Scavenging activity) | DPPH (mmol TE/g DW) | ORAC (μmol TE/g DW) | FRAP (mmol TE/g DW) | NO scavenging (%NO scavenging activity) |
|---|---|---|---|---|---|
| **TEE** | 14.62±3.46[a] | 129.57±41.01[a] | 1686.82±44.24[a] | 83.07±13.36[a] | 54.75±3.40[a] |
| **TME** | 11.32±0.90[ab] | 90.52±10.70[b] | 1076.22±44.83[b] | 79.33±12.21[a] | 52.58±5.37[ab] |
| **THE** | 9.55±0.89[b] | 69.57±10.52[c] | 1179.09±41.76[b] | 54.59±4.37[b] | 40.68±3.89[c] |
| **TAE** | 87.41±0.36[c] | 173.11±1.07[d] | 710.11±4.30[c] | 1901.20±16.09[c] | 37.05±4.06[c] |

Values are mean ± SD of three independent experiments. Different letters above the values indicate significant differences among samples in each assay ($p<0.05$). Dry weight (DW); Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk aqueous water extract (TAE); Trolox equivalent (TE)

**Table 2.** Total flavonoid and phenolic contents of *A. lebbeck* leaf extracts

| Sample | Total flavonoid content (mg QE/100g DW) | Total phenolic content (mg GAE/100g DW) |
|---|---|---|
| **TEE** | 1261.60±37.57[a] | 12750.00±272.43[a] |
| **TME** | 1069.09±64.44[b] | 13187.50±225.35[ab] |
| **THE** | 678.39±75.03[c] | 11375.00±165.36[a] |
| **TAE** | 626.80±27.09[c] | 15345.83±1025.35[c] |

Values are mean ± SD of three independent experiments. Different letters above the values indicate significant differences among in each assay ($p<0.05$). Dry weight (DW); Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk aqueous water extract (TAE); Quercetin equivalent (QE); Gallic acid equivalent (GAE)

***A. lebbeck* leaf extracts reduce the formation of mutagen**

In the antiformation of mutagen model, each sample was tested at the same dosage per plate: 375, 750, 1500, 3000, and 6000 μg/plate. In both strains, all concentrations of extracts exhibited histidine revertant colonies with a dose-response relationship for *S. typhimurium* strains TA98 and TA100. The *A. lebbeck* leaf extracts from ethanol, mix solvents, and hexane were active in antiformation of mutagen at moderate to strong activity, while *A. lebbeck* leaf aqueous water extract showed no to moderate active capability (**Table 4 and Figure 2**). According to these data, *A. lebbeck* leaf extracts might have a higher potential in inhibiting frameshifts mutation (TA98) (**Figure 2A**) than base-pair substitutions mutation (TA100) (**Figure 2B**) without metabolic enzyme activation.

**Antimutagenicity of *A. lebbeck* leaf extracts**

In the antimutagenicity model, each sample was tested at the same concentration per plate: 375, 750, 1500, 3000, and 6000 μg/plate. For both strains, all concentrations of the extracts were examined for dose-response

**Table 3.** Mutagenicity of *A. lebbeck* leaf extracts towards *S. typhimurium* strains TA98 and TA100

| Sample | Concentration (µg/plate) | No. of histidine revertant colony* /plate | |
|---|---|---|---|
| | | TA98 | TA100 |
| Negative control[a] | | 29.33±1.15 | 111.67±1.53 |
| TEE | 375 | 22.67±1.15 | 129.33±2.52 |
| | 750 | 20.67±2.52 | 97.67±4.93 |
| | 1500 | 24.00±2.65 | 113.67±1.53 |
| | 3000 | 27.33±1.53 | 104.67±2.52 |
| | 6000 | 26.67±2.52 | 133.67±6.11 |
| TME | 375 | 34.67±4.04 | 96.33±3.51 |
| | 750 | 37.33±2.08 | 102.00±10.58 |
| | 1500 | 23.67±1.53 | 124.00±6.56 |
| | 3000 | 20.33±3.06 | 97.33±6.03 |
| | 6000 | 27.67±2.52 | 145.67±5.77 |
| THE | 375 | 38.67±3.79 | 107.33±5.51 |
| | 750 | 35.00±4.58 | 112.67±6.03 |
| | 1500 | 45.00±2.00 | 102.00±5.57 |
| | 3000 | 24.33±3.51 | 127.33±9.02 |
| | 6000 | 27.00±1.00 | 96.33±1.53 |
| TAE | 375 | 28.67±2.52 | 109.67±3.06 |
| | 750 | 24.00±2.65 | 125.67±8.02 |
| | 1500 | 21.00±1.00 | 106.33±3.51 |
| | 3000 | 23.67±1.53 | 93.33±3.06 |
| | 6000 | 21.33±2.08 | 109.00±3.61 |

mean ± SD of six plates from at least three different experiments; *Number of histidine revertant colonies is represented and followed by criteria for evaluation of mutagenicity in the Ames test.; [a]Negative control refers to spontaneous mutation.; Nitrite-treated 1- aminopyrene (AP) refers to nitroaminopyrene (0.037 µg/plate) prepared from nitrite-treated 1-AP, which acts as standard mutagen in without metabolic enzyme activation system, was equal to 730.67 ± 9.07 $His^+$ revertant colony/plate (TA98) and 1842.00 ± 49.52 $His^+$ revertant colony/plate (TA100). Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk leaf aqueous water extract (TAE).

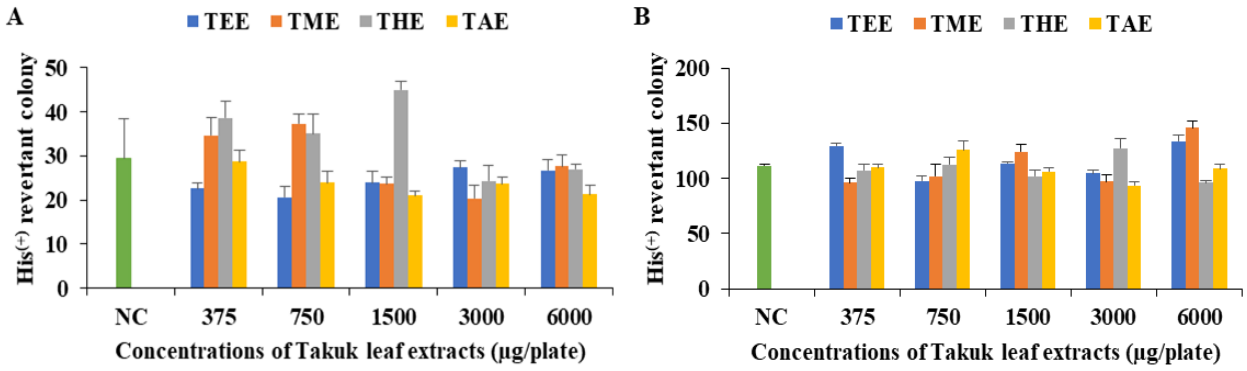


**Figure 1.** Mutagenicity of *A. lebbeck* leaf extracts towards *S. typhimurium* strains TA98 (A) and TA100 (B). Negative control (NC) refers to spontaneous mutation. Data are presented as mean ± SD of six plates from at least three different experiments. Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk leaf aqueous water extract (TAE).

relationships. Our results showed that all of the different solvent extracts of *A. lebbeck* leaf at concentrations of 375 - 6000 µg/plate showed percentage inhibition of ethanol, mix solvents, hexane, and aqueous water extracts at more than 60% and they are thus classified as strongly active antimutagenicity as shown in **Table 5** and **Figure 3**. According to these data, *A. lebbeck* leaf extracts might have a higher potential in inhibiting base-pair substitutions mutation (TA100) (**Figure 3B**) than frameshifts mutation (TA 98) (**Figure 3A**) without metabolic enzymes activation.

## Discussion

Phenolic compounds are regarded as active compounds and play a role in determining the antioxidant potential of vegetables and fruits. Fascinatingly, several scientific studies on *A. lebbeck* leaf have indicated that antioxidant activity is associated with both total phenolic content and flavonoids content[20, 43]. One of the most crucial mechanisms of action for antioxidant compounds is the neutralization of free radicals. The DPPH radical scavenging assay showed that *A. lebbeck* leaf extracts possessed a strong capability to scavenge the radicals, correlating with the ferric reducing antioxidant power values. The NO scavenging activity showed that *A. lebbeck* leaf extracts had moderate ability at scavenging the NO radical. The oxygen radical absorption capacity indicated a high potential for antioxidant capacity in *A. lebbeck* leaf extracts. Additionally, *A. lebbeck* leaf extracts had high amounts of total phenolic compounds and total flavonoids (**Table 1 and Table 2**). These results suggest that these leaf extracts may play a significant role in the ability to scavenge the radicals and reduce the potential of radical-induced oxidation and nitrosation in a chain reaction. Polyphenols and other phytochemicals in *A. lebbeck* leaves, such as flavonoids, carotenoids, saponins, and tannins, have been demonstrated to possess significant biological properties linked to antioxidant properties and the capacity to stimulate specific molecular pathways associated with the antioxidant response[20-21, 44]. The mutagenic potential of *A. lebbeck* leaf extracts was assessed using the Ames test without metabolic activation. Developed in 1973

**Table 4.** Antiformation of mutagen of *A. lebbeck* leaf extracts towards *S. typhimurium* strains TA98 and TA100

| Sample | Concentration (µg/plate) | No. of histidine revertant colony[#,*]/plate | | % Inhibition[**] | |
|---|---|---|---|---|---|
| | | TA98 | TA100 | TA98 | TA100 |
| Negative control[$] | | 48.80±2.31 | 101.33±2.89 | | |
| Nitrite-treated 1-AP[$$] | | 1668.40±66.93 | 1776.00±4.00 | | |
| TEE | 375 | 275.00±15.52 | 1022.00±17.69 | 84.76$^{a}$(S) | 45.02$^{a}$(M) |
| | 750 | 233.67±2.52 | 876.33±14.29 | 87.48$^{b}$(S) | 53.72$^{b}$(M) |
| | 1500 | 227.00±6.56 | 740.00±13.86 | 87.91$^{b}$(S) | 61.86$^{c}$(S) |
| | 3000 | 201.00±3.00 | 636.67±8.08 | 89.92$^{c}$(S) | 68.03$^{d}$(S) |
| | 6000 | 169.33±6.43 | 540.33±10.97 | 91.70$^{d}$(S) | 73.79$^{e}$(S) |
| TME | 375 | 253.67±29.67 | 945.67±10.60 | 86.16$^{a}$(S) | 49.58$^{a}$(M) |
| | 750 | 236.33±8.33 | 850.00±20.30 | 87.30$^{a}$(S) | 55.29$^{b}$(M) |
| | 1500 | 191.33±4.16 | 700.33±5.51 | 90.26$^{b}$(S) | 64.23$^{c}$(S) |
| | 3000 | 171.00±5.57 | 631.67±10.97 | 91.59$^{b}$(S) | 68.33$^{d}$(S) |
| | 6000 | 167.33±11.02 | 551.67±15.18 | 91.83$^{b}$(S) | 73.11$^{e}$(S) |
| THE | 375 | 739.33±31.07 | 1121.33±47.72 | 54.26$^{a}$(M) | 39.09$^{a}$(W) |
| | 750 | 380.67±8.08 | 844.00±20.00 | 77.82$^{b}$(S) | 55.65$^{b}$(M) |
| | 1500 | 302.67±4.16 | 666.33±13.58 | 82.94$^{c}$(S) | 66.26$^{c}$(S) |
| | 3000 | 204.33±7.51 | 521.33±14.05 | 87.04$^{d}$(S) | 74.92$^{d}$(S) |
| | 6000 | 213.33±7.02 | 345.33±13.43 | 88.81$^{d}$(S) | 85.43$^{e}$(S) |
| TAE | 375 | 1462.67±22.03 | 1580.00±4.00 | 6.74$^{a}$(N) | 11.70$^{a}$(N) |
| | 750 | 1256.33±17.79 | 1336.67±31.01 | 20.30$^{b}$(N) | 26.23$^{b}$(W) |
| | 1500 | 977.33±10.07 | 1255.00±27.07 | 38.62$^{c}$(W) | 31.11$^{c}$(W) |
| | 3000 | 873.67±19.40 | 1159.33±21.20 | 45.43$^{d}$(M) | 36.82$^{d}$(W) |
| | 6000 | 666.67±8.33 | 1018.67±24.11 | 59.03$^{e}$(M) | 45.22$^{e}$(M) |

[#]mean ± SD of six plates from at least three different experiments; [*]Number of histidine revertant colonies is represented and followed by criteria for evaluation of mutagenicity in the Ames test.; [$]Negative control refers to spontaneous mutation.; [$$]Nitrite-treated 1- aminopyrene (AP) refers to nitroaminopyrene (0.037 µg/plate) prepared from nitrite-treated 1-AP, which acts as standard mutagen in without metabolic enzyme activation system.; [**]Different letters above the percent inhibition values indicate significant differences among concentration groups ($p<0.05$).; (S) = strong inhibition.; (M) = moderate inhibition.; (W) = weak inhibition.; (N) = none inhibition.; Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk leaf aqueous water extract (TAE).

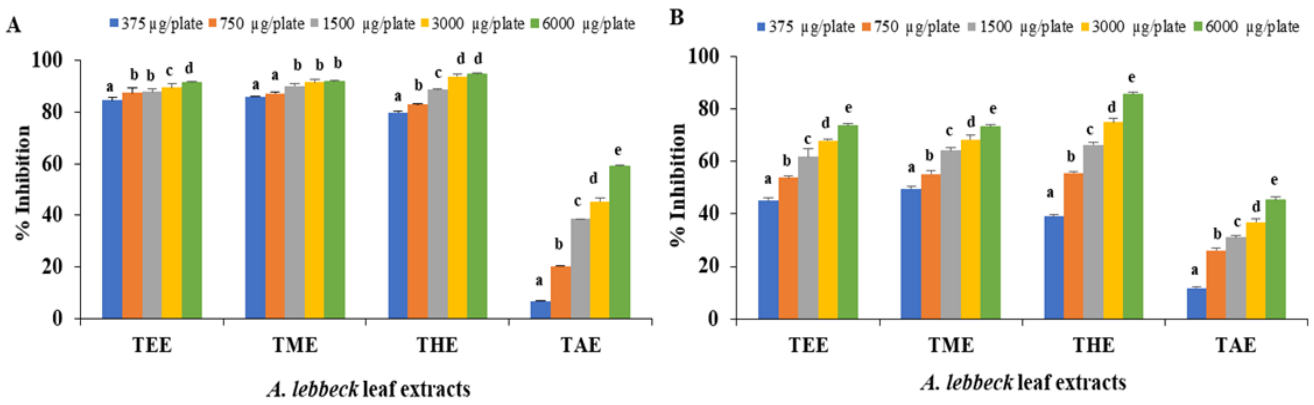


**Figure 2.** Antiformation of mutagen of *A. lebbeck* leaf extracts towards *S. typhimurium* strains TA98 (A) and TA100 (B). Different letters above the error bars indicate significant differences among concentration groups ($p<0.05$); Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk leaf aqueous water extract (TAE).

by Bruce N. Ames, the Ames test is a brief bacterial reversion assay that utilizes specially engineered mutants of *S. typhimurium* to assess the mutagenic properties of chemical compounds. This genotoxicity assay is faster and more cost-effective than traditional animal testing and tissue culture. For the mutagenicity testing, our findings indicated that there was no mutagenicity observed in any *A. lebbeck* leaf extracts towards TA98 and TA100 without metabolic activation (**Table 3 and Figure 1**). This event is consistent with the mutagenic activity test results in the different parts of the plant. According to Abdul-Hafeez et al. (2018)[2], they evaluated the mutagenic activity of *A. lebbeck* stem bark aqueous extract by the Ames test. Mutagenic testing in *S. typhimurium* (TA 98 and TA 100 strains) demonstrated that *A. lebbeck* extract did not induce the growth of histidine revertant colonies in both strains, indicating the absence of mutagenicity, since the number of histidine revertant colonies on mutagenicity testing was less than the number of spontaneous revertant colonies. A significant defensive mechanism against chemical mutagenesis involves the direct chemical interaction between an antimutagenic compound and a mutagen before causing DNA damage. In this study, we examined the antiformation of mutagen of *A. lebbeck* leaf extracts. The sample extracts were evaluated for their capacity to mimic the formation of carcinogens by nitrite salts in food proteins, wherein each sample was exposed to 1-aminopyrine and nitrite treatment before being introduced to tester strains. Each extract was tested at the same concentration per plate (375, 750, 1500, 3000, and 6000 µg/plate). All concentrations of each extract exhibited the revertant colony numbers with a dose-dependent manner in both *S. typhimurium* strains TA98 and TA100 (**Figure 2**). The *A. lebbeck* leaf ethanol extract, mix solvents extract, hexane extract and aqueous water extract could reduce the number of histidine revertant colonies at the stage preceding carcinogenesis (**Table 4**). It is associated with the biological antioxidant activity,

**Table 5.** Antimutagenicity of *A. lebbeck* leaf extracts towards *S. typhimurium* strains TA98 and TA100

| Sample | Concentration (µg/plate) | No. of histidine revertant colony[#,*]/plate | | % Inhibition[**] | |
|---|---|---|---|---|---|
| | | TA98 | TA100 | TA98 | TA100 |
| Negative control[$] | | 29.33±1.15 | 126.00±1.00 | | |
| Nitrite-treated 1-AP[$$] | | 730.67±9.07 | 2070.67±40.46 | | |
| TEE | 375 | 169.00±4.58 | 349.33±8.96 | 80.09$^{a}$(S) | 86.68$^{a}$(S) |
| | 750 | 156.67±2.08 | 283.33±3.06 | 81.84$^{a}$(S) | 89.18$^{a}$(S) |
| | 1500 | 126.33±5.51 | 236.33±2.52 | 86.17$^{ab}$(S) | 94.00$^{a}$(S) |
| | 3000 | 98.33±2.51 | 230.33±1.53 | 90.16$^{b}$(S) | 96.23$^{a}$(S) |
| | 6000 | 86.33±1.15 | 182.67±7.51 | 91.87$^{b}$(S) | 96.88$^{a}$(S) |
| TME | 375 | 182.67±4.04 | 385.00±2.65 | 78.14$^{a}$(S) | 88.52$^{a}$(S) |
| | 750 | 161.67±4.73 | 336.33±2.52 | 81.13$^{ab}$(S) | 91.91$^{a}$(S) |
| | 1500 | 135.33±3.51 | 242.67±3.06 | 84.89$^{bc}$(S) | 94.33$^{a}$(S) |
| | 3000 | 125.00±3.60 | 199.33±2.08 | 86.36$^{bc}$(S) | 94.63$^{a}$(S) |
| | 6000 | 111.67±1.52 | 186.67±3.21 | 88.26$^{d}$(S) | 97.09$^{a}$(S) |
| THE | 375 | 170.00±7.00 | 368.00±4.58 | 79.94$^{a}$(S) | 79.14$^{a}$(S) |
| | 750 | 148.33±5.13 | 224.67±4.51 | 83.03$^{ab}$(S) | 85.00$^{a}$(S) |
| | 1500 | 108.00±1.00 | 176.33±.6.66 | 88.78$^{b}$(S) | 86.97$^{a}$(S) |
| | 3000 | 71.67±0.58 | 164.67±1.53 | 93.96$^{c}$(S) | 90.40$^{a}$(S) |
| | 6000 | 66.00±2.65 | 127.67±2.52 | 94.77$^{c}$(S) | 92.46$^{a}$(S) |
| TAE | 375 | 278.33±4.51 | 531.67±4.51 | 64.50$^{a}$(S) | 87.56$^{a}$(S) |
| | 750 | 263.00±2.00 | 417.67±6.43 | 66.68$^{a}$(S) | 94.93$^{a}$(S) |
| | 1500 | 259.33±3.05 | 379.33±6.66 | 67.21$^{a}$(S) | 97.41$^{a}$(S) |
| | 3000 | 253.00±3.00 | 312.67±4.93 | 68.11$^{a}$(S) | 98.01$^{a}$(S) |
| | 6000 | 242.33±2.08 | 272.67±3.21 | 69.63$^{a}$(S) | 99.91$^{a}$(S) |

[#]mean ± SD of six plates from at least three different experiments; [*]Number of histidine revertant colonies is represented and followed by criteria for evaluation of mutagenicity in the Ames test.; [$]Negative control refers to spontaneous mutation.; [$$]Nitrite-treated 1- aminopyrene (AP) refers to nitroaminopyrene (0.037 µg/plate) prepared from nitrite-treated 1-AP, which acts as standard mutagen in without metabolic enzyme activation system.; [**]Different letters above the percent inhibition values indicate significant differences among concentration groups ($p<0.05$).; (S) = strong inhibition.; (M) = moderate inhibition.; (W) = weak inhibition.; (N) = none inhibition.; Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk leaf aqueous water extract (TAE).

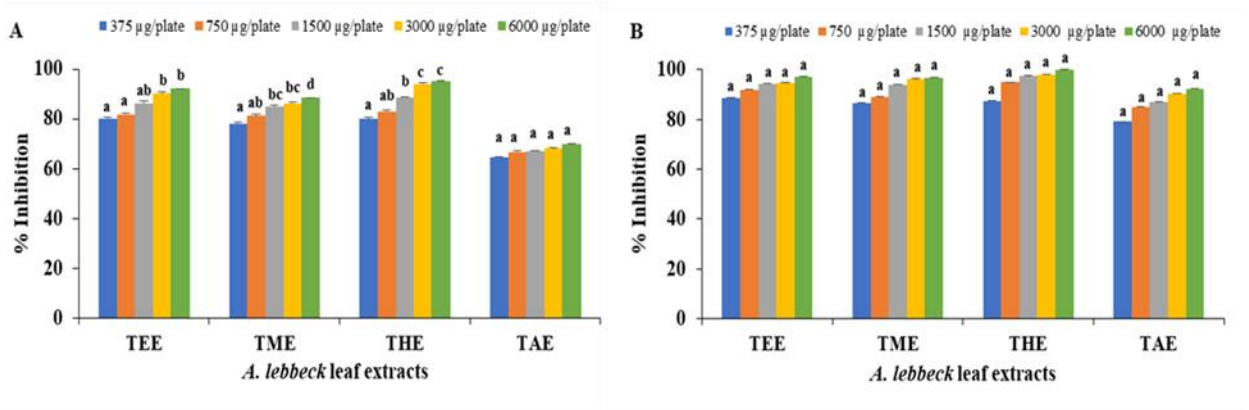


**Figure 3.** Antimutagenicity of *A. lebbeck* leaf extracts towards *S. typhimurium* strains TA98 (A) and TA100 (B). Different letters above the error bars indicate significant differences among concentration groups ($p<0.05$); Ta-kuk leaf ethanol extract (TEE); Ta-kuk leaf mix solvents extract (TME); Ta-kuk leaf hexane extract (THE); Ta-kuk leaf aqueous water extract (TAE).

which might hinder the role of AP-nitrite in the formation of direct mutagens. However, A. lebbeck leaf aqueous water extract showed less inhibition of mutagen formation compared to the results of other leaf extract samples at the same concentrations. Consequently, *A. lebbeck* leaf from ethanol extract, mix solvents extract, and hexane extract were active in antiformation of mutagen at moderate to strong inhibition, while *A. lebbeck* leaf aqueous water extracts showed no to moderate inhibition, depending on the concentrations of extracts. According to the available data, *A. lebbeck* leaf extracts might have a higher potential in inhibiting frameshifts (TA98) than base-pair substitutions (TA100) mutations without metabolic enzymes activation. This research demonstrated that phenolic compounds from various plant extracts can inhibit the formation of mutagens, thereby potentially reducing the risk of mutagen-induced diseases by antioxidants and helping to eliminate these oxidative products. Under typical cellular conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS)[12, 21, 45-47] found that doses of beta-carotene and selenium as covariates were significantly linked to the estimated intervention impact of antioxidants on gastrointestinal cancers. Antioxidants and natural products, including phenolic compounds, can neutralize the inhibitory effects of the formation of mutagens[48-49]. Consequently, the antiformation of mutagen in *A. lebbeck* leaf extracts may be linked to their direct action during the reaction of nitrite-treated 1-aminopyrene, as well as their antioxidant properties obtained from phenolic components and other bioactive compounds present in the extracts. Our study also demonstrated the antimutagenicity of *A. lebbeck* leaf extracts. The results suggest that these extracts have a strong potential antimutagenicity on AP-nitrite product mutagenesis, without metabolic activation, in *S. typhimurium* strains TA 98 and TA 100 (**Table 5** and **Figure 3**). The reduction in TA100 revertant colonies caused by nitrite-treated 1-aminopyrene indicates that *A. lebbeck* leaf extracts contain bioactive substances that disrupt the mutagenic metabolism of the final mutagen or carcinogen, form a complex of phenolic compounds with 1-nitropyrene, or neutralize the electrophilic

metabolites[50]. The findings of this study align with prior research indicating that phenolic compounds derived from plants are primarily effective against aromatic carcinogens, acting as inhibitors of mutagenesis in *in vitro* systems. Rahul et al. (2010)[16] reported that tri-O-glycoside, flavonols, kaempferol, albiziahexoside and quercetin were identified from the leaves of *A. lebbeck* and had the bioactive compounds of alkaloids, glycoside, tannins, saponins, flavonoids. These compounds in *A. lebbeck* leaf extracts may contribute to inhibiting the formation of mutagens and suppressing mutagenicity, aligning with our findings as illustrated in **Tables 4 and 5**. Additionally, plant phenolics have shown inhibitory effects against chemically induced carcinogenesis [48-49, 51-55], which is consistent with the results of antimutagenic activity of this study. A previous study demonstrated that *A. lebbeck* stem bark extracts showed detectable antimutagenic effect towards direct acting mutagens 2-nitrofluorene in TA98 as well as sodium azide in TA100[26]. Studies also suggest that phytochemicals found in *A. lebbeck* leaves can stimulate glucuronidation through UDP-glucuronosyltransferase. The glucuronidation pathway stands out as the primary route in phase II detoxification[56]. Phase II detoxification is a crucial process that helps to prevent the formation of DNA adducts, which are known to cause mutation and cancer due to exposure to toxins and carcinogens. This process is supported by enzymes such as glutathione-S-transferases that play a significant role in defending against mutagen/carcinogen-induced mutagenesis or carcinogenesis. This detoxifying enzyme has the ability to transform chemical carcinogens into less harmful or inactive metabolites by reducing the formation of DNA adducts[57]. It can be activated by numerous phytochemicals and bioactive components present in *A. lebbeck* leaf extracts. Consequently, the antioxidant compounds contained in the young leaf extracts of *A. lebbeck* may exert a protective role against mutation-related diseases like cancer, suggesting potential antiformation of mutagen as well as antimutagenicity for this plant.

## Conclusion

This study demonstrated that *A. lebbeck* leaf extracts have a good ability to scavenge radicals, reducing power activity, have high contents of total flavonoids and total phenolics, as well as having potent scavenging activity against the NO radical. The results of the Ames test without metabolic enzyme revealed that *A. lebbeck* leaf extracts showed no toxicity and no mutagenicity in *S. typhimurium* strains TA98 and TA100. In the antiformation of mutagen model, ethanol, mix solvents, and hexane leaf extracts at the highest concentrations strongly inhibited the formation of mutagens in both strains, while the aqueous water extract showed moderate activity in both of strains. In the antimutagenicity model, all extracts exhibited strong antimutagenic activity for both strains. Collectively, these findings suggest that the edible young leaves of *A. lebbeck* have antioxidant properties that can prevent the formation of mutagens and antimutagenicity against nitroaminopyrene direct-acting mutagenesis. Nevertheless, further research is required to examine other strains with metabolic enzyme systems according to OECD Guidelines No. 471, as well as in cell-based and animal studies for validating their other

genotoxicity properties. These findings provide preliminary data to indicate the safety of edible leaves of *A. lebbeck* and increase basic knowledge related to mutagenic or antimutagenic properties of Thai native edible plants in support of using this leaf as a natural alternative therapy to prevent the risk of mutation and cancer.

## Acknowledgment

This work was partially supported by the National Research Council of Thailand (NRCT32311/2020 and N21A661220/2023). Authors would like to thank the staff from the conservation area of the Electricity Generating Authority of Thailand, Srinakarind Dam, Kanchanaburi province, for providing the *A. lebbeck* leaves and gratefully appreciate the Plant Genetic Conservation Project initiated by Her Royal Highness Princess Maha Chakri Sirindhorn. We would like to express our gratitude to Asst. Prof. Dr. Thaya Jenjittikul for identifying and verifying the plant species. The authors are also grateful to the Institute of Nutrition, Mahidol University, Thailand, for supporting and providing the location and instruments used in this study. Special thanks to Medicinal Plant Research Institute, Department of Medical Sciences, Thailand, for the courtesy of providing the stock of tester strains used in the Ames test.

## Conflict of Interest

The authors have no conflicts of interest to declare.

## References

1. Brown TA. Genomes 2. 2nd ed. Oxford: Wiley-Liss Copyright ©. 2002.
2. Khader M, Bresgen N, Eckl PM. Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. J Ethnopharmacol. 2010;127(2):319-24.
3. Totsuka Y, Watanabe M, Lin Y. New horizons of DNA adductome for exploring environmental causes of cancer. Cancer Sci. 2021;112(1):7-15.
4. Xue Y, Wang L, Zhang Y, Zhao Y, Liu Y. Air pollution: A culprit of lung cancer. J Hazard Mater. 2022; 434:128937.
5. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001;411(6835):366-74.
6. Jiao Y, Li S, Wang X, Yi M, Wei H, Rong S. A genomic instability-related lncRNA model for predicting prognosis and immune checkpoint inhibitor efficacy in breast cancer. Front Immunol. 2022;13:929846.
7. Yoshioka KI, Kusumoto-Matsuo R, Matsuno Y, Ishiai M. Genomic Instability and Cancer Risk Associated with Erroneous DNA Repair. Int J Mol Sci. 2021;22(22):12254.
8. World Health Organization (WHO). Cancer [Internet]. 2022 [retrieved January 2023]. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer.
9. Upadhyay A. Cancer: An unknown territory; rethinking before going ahead. Genes Dis. 2020;8(5):655-61.
10. Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T. The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: A population-based modelling study. EClinicalMedicine. 2022;47:101404.
11. National Cancer Institute (NCI). Hospital-based cancer registry 2021 [Internet]. 2021

[retrieved january 2023]. Available from: https://www.nci.go.th/e_book/hosbased_2564/index.html.

12. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements. Aliment Pharmacol Ther. 2008;28(6):689-703.
13. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015;20(12):21138-56.
14. Gentscheva G, Karadjova I, Radusheva P, Minkova S, Nikolova K, Sotirova Y. Determination of the elements composition in *Sempervivum tectorum* L. from Bulgaria. Horticulturae. 2021;7(9):306.
15. Gentscheva G, Milkova-Tomova I, Nikolova K, Buhalova D, Andonova V, Gugleva V. Antioxidant activity and chemical characteristics of *Sambucus nigra* L. blossom from different regions in Bulgaria. Horticulturae. 2022;8(4):309.
16. Mihaylova D, Popova A. Phytochemicals of natural products: analysis and biological activities. Horticulturae. 2023;9(2):167.
17. Horn RC, Vargas VM. Antimutagenic activity of extracts of natural substances in the *Salmonella*/microsome assay. Mutagenesis. 2003;18(2):113-8.
18. Balkrishna A, Sakshi, Chauhan M, Dabas A, Arya V. A Comprehensive insight into the phytochemical, pharmacological potential, and traditional medicinal uses of *Albizia lebbeck* (L.) Benth. Evid Based Complement Alternat Med. 2022;2022:5359669
19. El-Ghany AES, Dora G, Abdallah RH, Hassan W, El-Salam EA. Phytochemical and biological study of *Albizia lebbeck* stem bark. J Chem Pharm Res. 2015;7(5):29-43.
20. Phoraksa O, Chimkerd C, Thiyajai P, udprasong K, Tuntipopipat S, Tencomnao T. Neuroprotective effects of *Albizia lebbeck* (L.) Benth. leaf extract against glutamate-induced endoplasmic reticulum stress and apoptosis in human microglial cells. Pharmaceuticals. 2023;16(7):989.
21. Praengam K, Muangnoi C, Charoenkiatkul S, Thiyajai P, Tuntipopipat S. Antioxidant and anti-inflammatory activity of aqueous fraction from *Albizia lebbeck* leaves. Int Food Res. J. 2017;24:1174-85.
22. Sridonpai P, Kongprapun P, Sungayuth N, Sukprasansap M, Chimkerd C, Judprasong K. Nutritive values and phytochemical compositions of edible indigenous plants in Thailand. Front Sustain Food Syst. 2022;6:870147.
23. Bobby MN, Wesely EG. *In vitro* anti– bacterial activity of leaves extracts of *Albizia lebbeck* Benth against some selected pathogens. Asian Pac J Trop Biomed. 2012;2:S859–62.
24. Goswami S, Malla S, Shrotri C. Phytochemical screening and antioxidant activity of extracts prepared from leaf and bark of *Albizia lebbeck*. Paper presented at: 1st International Conference on New Horizons in Pharmaceutical and Biomedical Sciences; 2013 January 12-13; Dehradun (UK), India.
25. Saha A, Ahmed M. The analgesic and anti-inflammatory activities of the extract of Albizia lebbeck in animal model. Pak J Pharm Sci. 2009;22(1):74-7.
26. Abdul-Hafeez EY, Karamova NS, Ilinskaya ON. Evaluation of mutagenic and antimutagenic potential of stem bark aqueous

extracts of eight trees by the bacterial reverse mutation assay. Ecological genetics. 2018;16(3):55-61.

27. Mlombo N, Dube Z, Makhubu F, Nxumalo H. Argemone ochroleuca phytochemicals and allelopathic effect of their extracts on germination of soybean. Int J Plant Biol. 2024;15(2):304-19.
28. Namkeleja H, Tarimo T, Ndakidemi P. Allelopathic effect of aqueous extract of *Argemone mexicana* L on germination and growth of Brachiaria *dictyoneura* L and *Clitoria ternatea* L. Am J Plant Sci. 2013;4(11):2138-47.
29. Kongkatitham V, Muangnoi C, Kyokong N, Thaweesest W, Likhitwitayawuid K, Rojsitthisak P. Anti-oxidant and anti-inflammatory effects of new bibenzyl derivatives from *Dendrobium parishii* in hydrogen peroxide and lipopolysaccharide treated RAW264.7 cells. Phytochem Lett. 2018;24:31-8.
30. Sukprasansap M, Pin-Am A, Kangsadalampai K. Thai desserts and snacks reduce the formation of mutagen, mutagenesis and their antioxidant activity. Poster session presented at: The 15th IUTOX International Congress of Toxicology (ICTXV); 2019 July 15-18; Honolulu, Hawaii, USA.
31. Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic Biol Med. 1993;14(3):303-11.
32. Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. J Agric Food Chem. 1996;44(11):3426-31.
33. Aktas N, Genc Y, Gozcelioglu B, Konuklugil B, Harput U. Radical scavenging effect of different marine sponges from mediterranean coasts. Rec Nat Prod. 2013;7(2):96-104.
34. Nantacharoen W, Baek SJ, Plaingam W, Charoenkiatkul S, Tencomnao T, Sukprasansap M. *Cleistocalyx nervosum var. paniala* berry promotes antioxidant response and suppresses glutamate-induced cell death via SIRT1/Nrf2 survival pathway in hippocampal HT22 neuronal cells. Molecules. 2022;27(18):5813.
35. Singsai K, Sakdavirote A, Wechpanishkitkul K, Moonsamai A. The comparison of phenolic compounds, flavonoids and antioxidant activities of the ethanolic extracts of shoots, leaves, fruits and seeds of *Leucaena leucocephala*. Naresuan Phayao J. 2020;13(3):66-73.
36. Moonnamarng N, Antiformation of mutagens and antimutagenicity of Thai berries extracts in vitro study. Poster session presented at: The 12th National Conference in Toxicology (NCT12); 2022 September 19; Bangkok, Thailand.
37. Sadabpod K, Kangsadalampai K, Tongyonk L. Antioxidant activity and antimutagenicity of hom nil rice and black glutinous rice. J Health Res. 2018;24(2):49-54.
38. Triteeradej N, Siritientong T, Tongyonk L. Mutagenicity and antimutagenicity of water extracts from gac fruit (*Momordica cochinchinensis* Spreng). J Health Res. 2017;30(6):387-92.
39. Wongwattanasathien O, Kangsadalampai K, Tongyonk L. Antimutagenicity of some flowers grown in Thailand. Food Chem Toxicol. 2010;48(4):1045-51.

40. Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. Mutat Res. 1983;113(3-4):173-215.
41. Tejs S. The Ames test: a methodological short review. Environ Biotechnol. 2008;4(1):7-14.
42. Calomme M, Pieters L, Vlietinck A, Vanden Berghe D. Inhibition of bacterial mutagenesis by citrus flavonoids. Planta Med. 1996;62(3):222-6.
43. Malla S, Shrotri C, Jain R. Antimicrobial, phytochemical and antioxidant screening of leaves and stem bark from *Albizia lebbeck* (L.). Int J Pharma Bio Sci. 2014;5:259-70.
44. Chulet R, Joseph L, George M, Pradhan P. Pharmacognostic standardization and phytochemical screening of *Albizzia lebbeck*. J Chem Pharm Res. 2010;2(1):432-43.
45. Król M, Kepinska M. Human nitric oxide synthase-its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and crdiovascular diseases. Int J Mol Sci. 2020;22(1):56.
46. Long S, Piao X. Effects of dietary *Forsythia suspensa* extract supplementation to lactating sows and nursery pigs on post-weaning performance, antioxidant capacity, nutrient digestibility, immunoglobulins, and intestinal health. J Anim Sci. 2021;99(8):skab142.
47. Wang DH, Wang MY, Shen WH, Yuan JF. Analysis of chemical compounds and toxicological evaluation of *Forsythia suspensa* leaves tea. Food Sci Biotechnol. 2021;30(2):305-14.
48. Makhafola TJ, Elgorashi EE, McGaw LJ, Verschaeve L, Eloff JN. The correlation between antimutagenic activity and total phenolic content of extracts of 31 plant species with high antioxidant activity. BMC Complement Altern Med. 2016;16(1):490.
49. Słoczyńska K, Powroźnik B, Pękala E, Waszkielewicz AM. Antimutagenic compounds and their possible mechanisms of action. J Appl Genet. 2014;55(2):273-85.
50. de Mejía EG, Castaño-Tostado E, Loarca-Piña G. Antimutagenic effects of natural phenolic compounds in beans. Mutat Res. 1999;441(1):1-9.
51. Akram M, Riaz M, Wadood A, et al. Medicinal plants with anti-mutagenic potential. Biotechnol Biotechnol Equip. 2020;34(1):309-18.
52. Newmark HL. Plant phenolics as inhibitors of mutational and precarcinogenic events. Can J Physiol Pharmacol. 1987;65(3):461-6.
53. Krizková L, Chovanová Z, Duracková Z, Krajcovic J. Antimutagenic *in vitro* activity of plant polyphenols: pycnogenol and *Ginkgo biloba* extract (EGb 761). Phytother Res. 2008;22(3):384-8.
54. Sarac N, Şen B. Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of *Liquidambar orientalis Mill. var. orientalis*. Ind Crops Prod. 2014; 53:60–4.
55. Snijman PW, Swanevelder S, Joubert E, Green IR, Gelderblom WC. The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*): some dose-response effects on mutagen activation-flavonoid interactions. Mutat Res. 2007;631(2):111-23.
56. Hodges RE, Minich DM. Modulation of metabolic detoxification pathways using foods and food-derived components: a scientific review with clinical application. J Nutr Metab. 2015; 2015:760689.

57. Sheweita SA. Narcotic drugs change the expression of cytochrome P450 2E1 and 2C6 and other activities of carcinogen-metabolizing enzymes in the liver of male mice. Toxicology. 2003;191(2-3):133-42.