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Research Article

Chemical Constituents, Antioxidant Property, Cytotoxicity and Genotoxicity of *Tiliacora Triandra*

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ABSTRACT

Tiliacora triandra has long been used as a traditional medicine and in cuisine in Thailand and neighboring countries. Leaf samples were randomly collected and extracted with methanol. The extracts were chemically analyzed by gas chromatography-mass spectrometry (GC-MS), and free radical scavenging activities were assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. After drying and re-dissolving the extracts in dimethyl sulfoxide (DMSO), cytotoxicity and genotoxicity tests were performed using lymphocytes and HeLa cells. The major components found in all extracts, with yields of 8.59-26.29%, were vitamin E, phytol and 1-cyclohexenylacetic acid. At a concentration of 8.4 mg/ml, the methanol extract reduced the concentration of free radical DPPH (DPPH•) to 50% (EC_{50}). Lymphocyte viability was 72.78% when treated with 10 mg/ml extract, a concentration that significantly affected DNA ($p<0.05$). The viability of HeLa cells was 50% (IC_{50}) when treated with 0.41 mg/ml; this concentration also significantly affected DNA ($p<0.05$). The extracts showed good antioxidant activity, in agreement with their high content of vitamin E, and significantly affected the stability of lymphocyte and HeLa DNA, though without major toxicity at the cellular level. These results reveal guidelines with regard to consumption of this plant, which is particularly common in Thailand.

Keywords: antioxidant, comet assay, DPPH assay, toxicity, *Tiliacora triandra*

INTRODUCTION

Tiliacora triandra (Colebr.) Diels, called *yanang* in Thai, is one of the most important vegetables used in Thai cuisine. Indeed, the aqueous extract of its leaves has long been used in the preparation of Thai dishes, especially in northeastern-style cooking, such as in *sup no mai* (spiced bamboo shoots), *kaeng no mai* (bamboo shoot soup), and *kang kee lek* (senna leaf curry). Given its long history of consumption and use as a traditional medicine, *T. triandra* has been thought to be safe for humans. Medicinal plants are a major chemical resource for medicines, cosmetics, perfumes and household products worldwide, and *T. triandra* is one such plant. The species is a member of a genus of flowering plant native to Southeast Asia and is particularly used in the cuisines of northeast Thailand and the Lao People's Democratic Republic (LPDR)[1].

Currently, Thai population consumes *T. triandra* as a part of the diet and also uses it as a traditional medicine; accordingly, the plant may contain compounds that could have enormous potential for treating human diseases. Previous researches on other plants have shown that the chemical composition of various extracts can be successfully determined using gas chromatography-mass spectrometry (GC-MS), and this method has been well accepted for phytochemical identification[2,3,4,5,6]. Many chemical compounds found in nature are beneficial to human health, reducing the need to generate synthetic agents, which can sometimes be difficult, time-consuming and/or expensive. However, more detailed studies of the compounds present in this plant should be performed to obtain information related to their safety and efficacy. Given its usage, the antioxidant activity and toxicity of *T. triandra* need to be evaluated.

Antioxidants play important roles as molecules that reduce the risk of chronic diseases, such as cancer and heart disease, and plant-sourced antioxidants include vitamins C and E and beta-carotene. A rapid, simple and inexpensive method for measuring antioxidant activity is based on the reduction of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•)[7, 8].

Additionally, before a product can be approved for use in humans, including food and medicinal plants, it must be thoroughly tested for toxicity. Single-cell gel electrophoresis, also known as the comet assay, is commonly used to assess the cytotoxicity and genotoxicity of compounds and mixtures. This method combines the simplicity of biochemical techniques that detect DNA

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Figure 1: The characteristics of *Tiliacora triandra* showing its vine, leaves and fruits.

Table 1: The absorbance and percentage of free radical scavenging by various concentrations of *T. triandra* extract.

Extract concentration (mg/ml)	Absorbance		Average absorbance	DPPH scavenging activity (%)
	Rep. 1	Rep. 2		
0.625	1.149	1.130	1.140	3.76
1.250	1.082	1.012	1.047	11.57
2.500	0.980	0.863	0.922	22.17
5.000	0.743	0.604	0.674	43.12
10.000	0.545	0.243	0.394	66.72

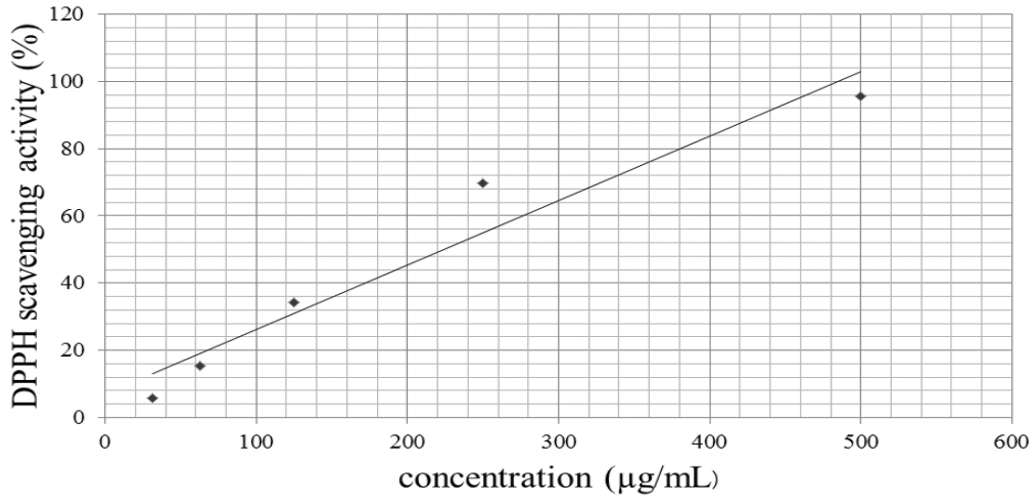

Figure 2: Percentage of DPPH scavenging activity (% inhibition) of *Tiliacora triandra* extract at different concentrations.

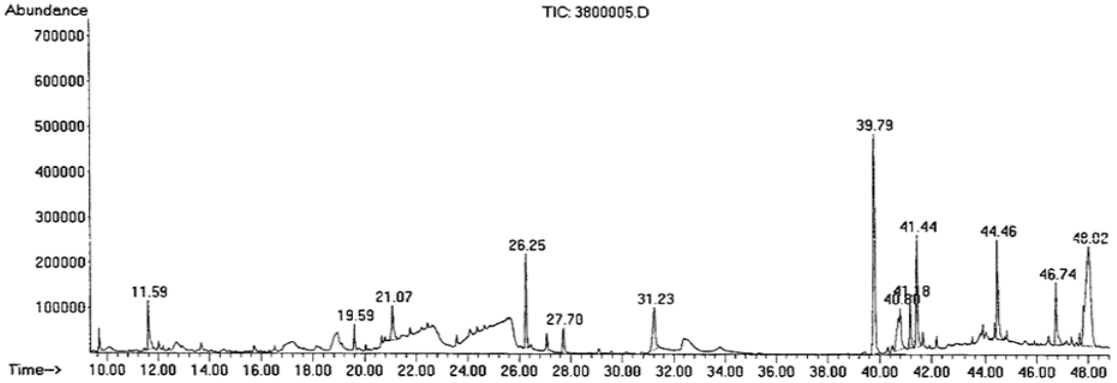

Figure 3: The total ionic chromatogram of a methanol crude extract of *Tiliacora triandra* leaves.

breaks (strand breaks and incomplete excision repair sites), alkali-labile sites and cross-linking with the single-cell approach typical of cytogenetic assays to measure DNA damage and repair at the level of individual cells (single-cell gel electrophoresis (SCG)). As fragments of DNA move toward the anode to form a comet tail as the frequency of DNA breaks increase[9], a longer tail represents a greater amount of DNA fragmentation and a greater level of genetic damage. This assay has been successfully applied to a range of phylogenetically diverse organisms, including mammals and lower- and higher-order plants[10]. Previous studies have employed a variety of physical and chemical agents during different life stages and in multiple cell types under both laboratory and field conditions, and the results have led to many discoveries reported in the literatures. For example, the comet assay was used to detect damage in human cells (MRC5CV1), leading to an understanding of the relevance of mutagenesis following exposure to environmental, mutagenic and carcinogenic polycyclic aromatic hydrocarbons and their reactive metabolites[11]. Rodrigues et al.[12] applied the comet assay and a micronucleus test in HTC rat hepatoma cells exposed to *Allium cepa* to genetically evaluate industrial effluent from an oil refinery. In addition, the comet assay is being increasingly used for genotoxicity testing of industrial chemicals, pharmaceuticals, and agrochemicals, such as herbicides and pesticides. The comet assay can also be used to support drug development as a predictor of mechanism of action, and it is becoming a common method for genotoxicity testing of potential medicinal plants[13].

Previously, some aspects of the most common species of *T. triandra* grown in Thailand and LPDR were assessed in acute and subchronic toxicity studies, with an aqueous extract causing no subchronic toxicity in male and female rats[14]. Singthong et al.[1] also studied *T. triandra* and reported some physicochemical characteristics of the polysaccharide gum extracted with water, indicating that it is a monosaccharide (xylose) with substantial amounts of other neutral sugars. The methanol extract of *T. triandra* reportedly has the highest antioxidant activity, and the phytochemicals present include alkaloids, flavonoids, tannins and saponins. In addition, this antioxidant activity has potential utility for the prevention of disease[15].

Although some research has been performed, the antioxidant activity and toxicity (including cytotoxicity and genotoxicity) of the long-used juice of *T. triandra* have not been intensively studied. Therefore, to confirm the safety of leaf extracts of this plant, we examined the antioxidant activity, cytotoxicity and genotoxicity using normal human leukocytes and the HeLa carcinoma cell line.

MATERIALS AND METHODS

Plant materials

Mature leaves of *Tiliacora triandra* were collected from three individual plants grown in different provinces of Thailand for the study of phytochemical components and toxicity.

Preparation of chemical extracts

To prepare leaf extracts, the native plant samples were rinsed with water and air-dried until the water had evaporated from the leaves. A 20-g sample was then ground into a powder, mixed with 125 ml of methanol (analytical grade) and filtered through filter paper at room temperature. Next, 80 ml of the filtrate (crude extract) was obtained and stored at -20°C until use.

Evaluation of antioxidant activity using the DPPH radical scavenging method

The free radical scavenging of different concentrations of leaf extract and a standard trolox solution in methanol was measured based on their ability to react with the stable DPPH radical (DPPH•) following Garcia et al.[7] and Shekhar and Anju[8]. The diluents used were 10, 5, 2.5, 1.25 and 0.625 mg/ml. A methanol DPPH• (2 mM) solution was prepared, and 200 µl was added to each tube of extract. The mixtures were shaken and allowed to stand in the dark at room temperature for 30 min, and the absorbance at 515 nm was then measured using a spectrophotometer. The experiment was performed in duplicate. A low absorbance of the reaction mixture indicated a high free radical scavenging activity. The concentration of sample required to scavenge 50% of the DPPH• (EC_{50}) was calculated from the inhibition curve. The percentage of DPPH scavenging activity (% inhibition) was calculated using the following equation:

$$\% \text{ inhibition} = 1 - (A_{sample} / A_{control}) \times 100$$

where A_{sample} is the absorbance in the presence of the test or standard sample and $A_{control}$ is the absorbance of the control reaction without the test or standard sample.

Gas chromatography-mass spectrometry (GC-MS) analysis and chemical identification

A GC-MS analysis of the crude extracts was performed using an Agilent Technologies GC 6890 N/5973 inert mass spectrometer combined with a capillary column (30.0 m × 250 µm × 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1 ml/min. The injection and mass-transferred line temperature was set to 280°C. The oven temperature was programmed to shift from 70°C to 120°C at 3°C/min, then was held isothermally for 2 min, and then increased to 270°C at 5°C/min. A 1-µl aliquot of the crude extract was injected in split mode. The relative percentage of the crude constituents was expressed as a percentage using peak area normalization. Component identification was performed by comparing the obtained mass spectra with the reference compounds in the Wiley 7N.1 library.

Cell line culture

The HeLa (human cervical carcinoma) cell line used in this study was kindly provided by Prof. Dr. Thomas Liehr (Institute of Human Genetics, University of Jena, Germany). The cells were cultured in liquid medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin at 37°C for 2 days. The cells were washed with phosphate-buffered saline (PBS) and harvested by trypsinization, and cell counts were performed using a hemocytometer with trypan blue staining. For further cytotoxic and genotoxic tests, a cell suspension was prepared at a concentration of 4-6x10^5 cells/ml of DMEM.

Lymphocyte isolation and preparation

Table 2: The chemical constituents of *Tiliacora triandra*, as determined by GC-MS

RT (min)	Common name	IUPAC	Formula	Relative content (%)
11.587	5-Hydroxymethyl-2-furancarboxaldehyde	5-(hydroxymethyl)furan-2-carbaldehyde	$C_6H_6O_3$	4.082
19.594	2,6-Dimethyl-3-(methoxymethyl)-benzoquinone	2-(methoxymethyl)-3,5-dimethyl-1,4-benzoquinone	$C_{10}H_{12}O_3$	1.490
26.245	Neophytadiene	7,11,15-trimethyl-3-methylidenehexadec-1-ene	$C_{20}H_{38}$	6.226
31.230	Hexadecanoate or Palmitic acid	hexadecanoic acid or tetradecanoic acid	$C_{16}H_{32}O_2$	5.462

Table 3: The chemical constituents of *Tiliacora triandra*, as determined by GC-MS (cont.)

RT (min)	Common name	IUPAC	Formula	Relative content (%)
39.791	Phytol	(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	19.566
40.806	Oleic acid	(9Z)-octadec-9-enoic acid	$C_{18}H_{34}O_2$	7.370
41.441	1-Cyclohexenylacetic acid	2-(cyclohexen-1-yl)acetic acid	$C_8H_{12}O_2$	8.587
44.458	Oleamide	(9Z)-octadec-9-enamide	$C_{18}H_{35}NO$	7.634
48.023	Vitamin E (α-Tocopherol)	(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1-benzopyran-6-ol	$C_{29}H_{50}O_2$	26.289

Anonymous blood samples were received from a blood bank collected in heparinized collection tubes. Lymphocytes were isolated by adding red blood cell lysis buffer and then centrifuging to initiate hemolysis. The collected lymphocytes were re-suspended in PBS. The cells were counted, and a suspension was prepared at a concentration of 4-6x10^5 cells/ml of RPMI.

In vitro assay for cytotoxicity with hemocytometer cell counting

The cell suspension was portioned into 1.5-ml microtubes (500 µl per tube) and incubated with different concentrations of the plant extract (50 µl per tube) at 37°C for 4 hr (lymphocytes) or 24 hr (HeLa cells). Untreated cells (negative control) were incubated in culture medium only. Positive control cells were incubated with 100 µM of H_2O_2 for 15 min. Additionally, DMSO was tested to measure its toxicity. Each experiment was performed in duplicate. After completed incubation, viable cells were count on hemocytometer with trypan blue stain and calculated for viability percentage using the following equation:

cell viability (%) = (average viable of treated cells/average viable negative control cells) × 100.

The percentage of viability was plotted against the concentration tested. The concentration of extract that produced 50% cell death (IC_{50}) was calculated from the viability curve.

In vitro assay for genotoxicity testing using the comet assay

Cells were treated as in the cytotoxicity test but were treated with plant extract at the IC_{50} concentration or at highest concentration in case of IC_{50} value is not available. The comet assay was performed according to the method described by Singh et al.[16], with slight modifications. After treatment of the extracts, cell pellets were obtained by centrifugation. The cells were then re-suspended in 40 µl of PBS and 100 µL of 0.5% low melting point agarose (LMA). The cell mixture was dropped onto slides that had been precoated with 1% normal melting point agarose, covered with cover slips (22 mm x 50 mm), and stored at 4°C for 10 min. The cover slips were removed and the slides were submerged in a lysis solution (8 M NaCl, 0.6 M EDTA pH 8, 0.2 M tris, 0.1% triton X-100) for at least 1 hr. The slides were then soaked in electrophoresis buffer (6 mM EDTA pH 10, 0.75 M NaOH) for 40 min, and electrophoresis was performed for 25 min at 26 V/300 mA at 4°C. After electrophoresis, the slides were immediately neutralized three times with 0.4 M Tris buffer (pH 7.5) for 5 min each. The slides were then stained with 1 µg/ml ethidium bromide (60 µl per slide) overnight at 4°C in the dark, and images were obtained using an image analysis system (Isis) attached to a fluorescence microscope (Nikon, Japan). Images of 150 cells per experiment were analyzed using ImageJ software (http://imagej.nih.gov/ij) and statistically analyzed by GraphPad Prism software (http://www.graphpad.com/scientific-software/prism).

The length and fragment intensity of the tail relative to the head is directly proportional to the amount of DNA damage[17].

RESULTS AND DISCUSSION

Evaluation of antioxidant activity

A methanol extract of *T. triandra* (Fig. 1) leaves was evaluated for antioxidant activity using the DPPH scavenging assay. For extract concentrations of 0.625, 1.250, 2.500 and 10.000 mg/ml, the average absorbance at 515 nm was 1.140, 1.047, 0.922, 0.674 and 0.394, respectively, and the percentage of DPPH scavenging was 3.76%, 11.57%, 22.17%, 43.12% and 66.72%, respectively. These experimental results are compiled in

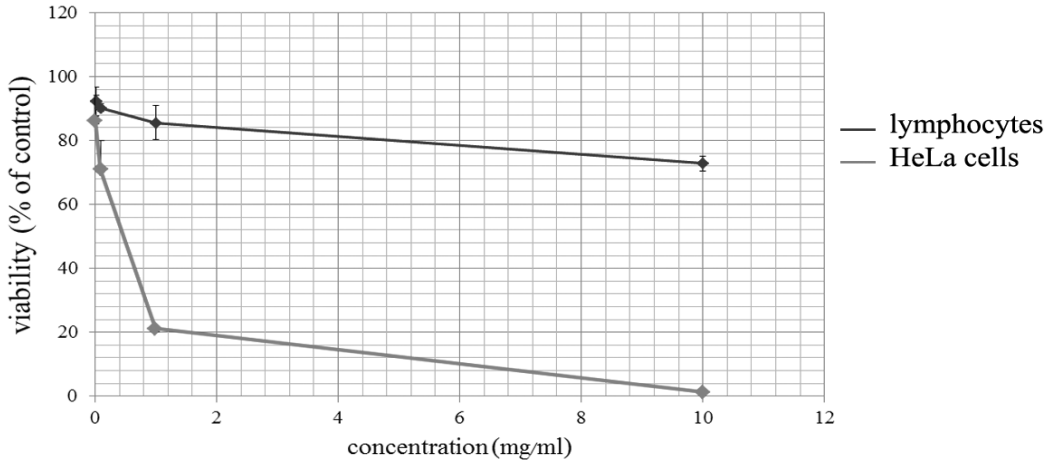

Figure 4: The cytotoxicity of the *Tiliacora triandra* extract toward lymphocytes and HeLa cells.

Table 1. As shown in Fig. 2, the percentage of DPPH scavenging and the extract concentration were plotted in a graph to identify the EC_{50} value with regard to antioxidant effects, which was found to be 8.4 mg/ml.

Phytochemical studies

Phytochemical screening of methanol crude extracts of *T. triandra* revealed the presence of various hydrocarbon compounds. The total ionic chromatograph (TIC) used to identify the compounds is shown in Fig. 3. The nine identified chemical compounds and their relative contents are provided in Table 2. Vitamin E (α-tocopherol) was the most prominent of the compounds, accounting for 26.29% of the identified compounds, which was followed by phytol, at 19.57%; the other compounds were present at concentrations less than 10% (Table 2).

Cytotoxicity

In vitro cytotoxicity testing using carcinoma cell lines has been successfully applied to identify the toxic and biological properties of plant extracts[18]. In this experiment, cytotoxic effects of the plant extract were defined as a loss of membrane activity in cells treated with trypan blue dye. The experiments indicated a clear dose-dependent cytotoxic effect of the *T. triandra* leaf extract on both lymphocytes and HeLa cells.

As the lymphocyte viability was 72.78% when treated with the highest concentration, 10 mg/ml extract, no IC_{50} value could be calculated. However, the viability of HeLa cells was 50% (IC_{50}) when treated with 0.41 mg/ml extract. The correlation between the viability and the extract concentration is shown in Fig. 4.

Genotoxicity

As there was no IC_{50} value for lymphocytes, the highest concentration (10 mg/ml) of the leaf extract was used for the comet assay. This concentration significantly affected DNA (p<0.05), as shown in Fig. 5. Evidence of DNA damage in the lymphocytes treated with the *T. triandra* extract was observed with a long tail and a broken head (b) compared to the untreated negative control (a). In contrast, the lymphocytes treated with DMSO did not show any significant DNA damage (Fig. 6). For HeLa cells, the IC_{50} of the *T. triandra* extract was selected for use in the comet assay, with significant DNA damage (p<0.05), as shown in Fig. 5(c). The median of the Olive tail moment for the DNA of lymphocytes and HeLa cells is plotted on the graph shown in Fig. 6.

General discussion

Tiliacora triandra has long been used as a traditional medicine and in cuisine in Thailand. Although there have been several studies on this plant, there is no information to date on its toxicity at the cellular and genomic levels. Previous studies on *T. triandra* have focused on its alkaloids[19] and the isolation of an anti-malarial active compound[20] as well as the toxicity of the aqueous extract in rats, with the results suggesting that the plant does not cause acute or subchronic toxicity in either male or female rats[14]. Other research includes the identification of polyphenolic compounds and colorants, the extraction and physicochemical characterization of the polysaccharide gum[1], the phytochemical screening and evaluation of the flavonoid content and assessment of the antioxidant activity of leaf extracts[15], the phytochemical screening and assessment of antioxidant and antimutagenic activities of selected edible Thai plant extracts, including an extract of *T. triandra*[21], and an evaluation of the bioactive compounds and encapsulation of the leaves[22]. The most recent study published in 2015 showed that *T. triandra*, an anti-intoxication plant, improved memory impairment, neurodegeneration, cholinergic function, and oxidative stress in the hippocampus of ethanol-dependent rats [23]. To the best of our knowledge, the present study is the first to address the toxicity of a *T. triandra* leaf extract for an in-depth evaluation of toxicity at the cellular and genomic levels.

With regard to the antioxidant activity of the *T. triandra* leaf extract, the results indicated good antioxidant activity, with a strong ability to decrease the concentration of DPPH• (with an EC_{50} of 8.4 mg/ml), indicating that the extract scavenges free radicals. Furthermore, the identification of economically beneficial chemicals present in *T. triandra* is important for global research and may also be beneficial for providing a growing population with supplements from natural sources. Such findings can also contribute to data on medically effective food plants and provide a basis for further research.

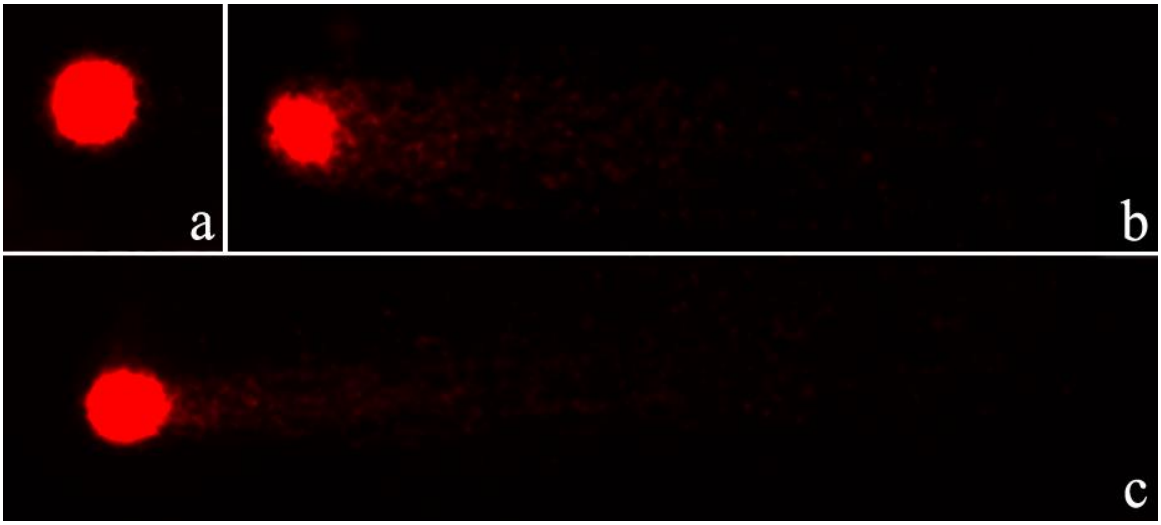

Figure 5: DNA comets of lymphocytes (a-b) untreated (negative control) (a) and treated with 10 mg/ml of the plant extract (b), indicating significant DNA damage ($p<0.05$), and HeLa cells treated with 0.41 mg/ml (the IC_{50}) of the plant extract (c), indicating significant DNA damage ($p<0.05$) (40x).

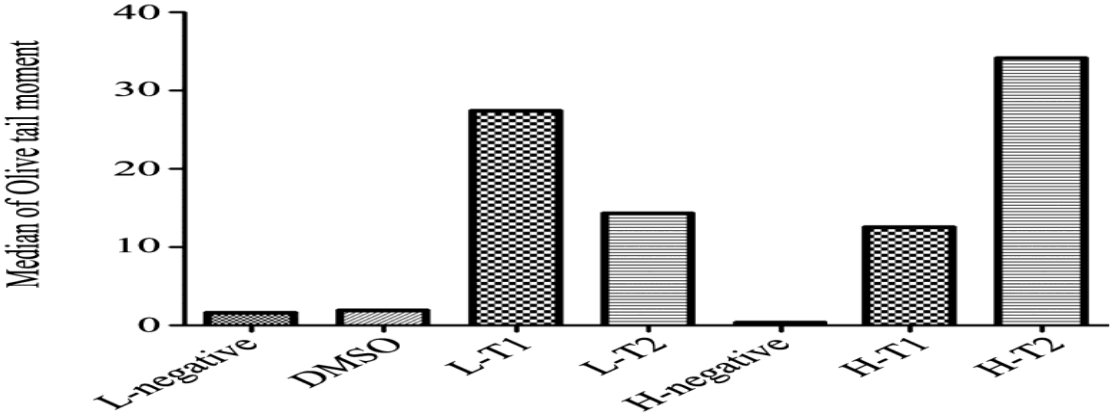

Figure 6: The median of Olive tail moment values of DNA from lymphocytes treated with the plant extract (L-T1, L-T2) compared to negative control cells (L-negative) not treated with any chemical and lymphocytes treated with DMSO alone, as well as HeLa cells treated with the plant extract (H-T1, H-T2) compared to negative control (H-negative) cells not treated with any chemicals.

In Thailand, the leaves of *T. triandra* have been used for the treatment of several ailments as well as in Thai cuisine, and it is possible that the leaves contain chemical constituents with enormous potential as antioxidants and with other activities. The chemicals found at relatively high levels in the leaves were vitamin E, phytol and 1-cyclohexenylacetic acid (26.29, 19.57 and 8.59%, respectively). The other components included oleamide and oleic acid (total 7.63 +7.37%), hexadecanoate or palmitic acid (5.46%), neophytadiene (6.23%), 2,6-dimethyl-3-(methoxymethyl)-benzoquinone (1.49%), and 5-hydroxymethyl-2-furancarboxaldehyde (4.08%). Several of these chemicals are well known to be useful in humans, as described below.

Vitamin E is fat-soluble and is found in many foods including vegetable oils, cereals, meat, poultry, eggs, fruits, vegetables, and wheat germ oil. It is also available as a supplement. Although vitamin E deficiency is rare, it can occur in people with certain genetic disorders and in premature infants with a very low birthweight. Additionally, vitamin E is used to treat many diseases or for preventing diseases of the heart and blood vessels, diabetes and its complications and cancer (particularly lung and oral cancer in smokers, colorectal cancer and colon polyps, and gastric, prostate, and pancreatic cancers) as well as diseases of the brain and nervous system, including Alzheimer's disease. This vitamin is also used to reduce unwanted side effects of drugs, such as hair loss in people taking doxorubicin and lung damage in people treated with amiodarone. Some people apply vitamin E to their skin to keep it from aging and to protect against the effects of chemicals used in cancer chemotherapy (http://www.webmd.com/vitamins-supplements/ingredientmono-954-vitamin%20e.aspx?activeingredientid=954&activeingredientname=vitamin%20e).

Phytol is an aromatic ingredient used in many fragrance compounds and is found in both cosmetic and non-cosmetic products. In the field of medicine, phytol has demonstrated antinociceptive and antioxidant activities as well as anti-inflammatory and antiallergic effects. Recent studies have revealed that phytol is an excellent immunostimulant, superior to a number of commercial adjuvants in terms of long-term memory induction and the

activation of both innate and acquired immunity. Additionally, phytol and its derivatives have no cumulative inflammatory or toxic effects, even in immunocompromised mice. Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus*[24].

Oleamide (7.63%) + oleic acid (7.37%) in *T. triandra* is produced endogenously from oleic acid, and the fatty acid was previously shown to be a protective agent against scopolamine-induced memory loss and was suggested to be a useful chemopreventive agent for Alzheimer's disease[25]. Oleamide occurs naturally in the bodies of animals, including humans, and accumulates in the cerebrospinal fluid during sleep deprivation, likely to induce sleep activity. Intracerebroventricular and intraperitoneal administration of oleamide reportedly induced sleep in rats, and the hypnogenic effects of oleamide are not related to changes in blood pressure, heart rate, or body temperature[26].

Hexadecanoate, also known as palmitic acid, which was found in *T. triandra* (5.462%), is the most common (saturated) fatty acid in animals, plants and microorganisms. It is used to produce soaps, cosmetics, and release agents and is widely used as a lubricant and as an additive in industrial preparations. Palmitic acid is also used in the manufacture of metallic stearates, pharmaceuticals, soaps, cosmetics, and food packing and is used as a softener, accelerator/activator and dispersing agent in rubber (http://www.chemicalland21.com/industrialchem/organic/PALMITIC%20ACID.htm).

Neophytadiene, also found in *T. triandra* (at 6.226%), is an enzyme inhibitor that contains 15% hexanes (http://www.scbt.com/datasheet-208090-neophytadiene.html). It is used as additive for electronic cigarettes because it can improve the aroma and evaporation rate. Liquid cigarettes contain from 25 to 99.9% atomized liquid by weight and from 0.01% to 75% neophytadiene by weight (World Intellectual Property Organization (CH), http://patentscope.wipo.int/search/en/detail.jsf?docId=WO201014 5469).

2,6-Dimethyl-3-(methoxymethyl)-benzoquinone, which is identical to 2-(methoxymethyl)-3,5-dimethyl-1,4-benzoquinone, 2-(methoxymethyl)-3,5-dimethyl-1,4-benzoquinone and 2,6-dimethyl-3-methoxymethyl-p-benzoquinone, was found at a concentration of 1.490% in *T. triandra*. There are currently no medication-related documents associated with this substance.

These above-mentioned substances may be useful for human health but are found in small amounts in the *T. triandra* extract. Two toxic substances were also found to be present in the *T. triandra* leaf extract.

One compound, 1-cyclohexenylacetic acid, has not been fully investigated for toxicity; however, it is not added to foods or drugs and is used only in the laboratory. Regardless, if the substance comes into contact with the eyes, it is recommended that they be immediately flushed with plenty of flowing water for 10 to 15 minutes while holding the eyelids apart (https://www.biosynth.com/downloadPDF/MSDS_W-107801_1000_ EN.pdf).

Hydroxymethyl-2-furancarboxaldehyde (HMF) induces HepG2 DNA damage at concentrations from 7.87 to 25 mM, as based on the comet assay. The result indicates that HMF has a weak genotoxic effect in these cells, though it was suggested that the damage could most likely be repaired[27].

These two substances may have significantly affected the stability of the lymphocyte DNA, as indicated by the comet assay, when evaluated at the highest concentration (10 mg/ml) (Figs. 5 and 6). Additionally, treatment with the leaf extract significantly damaged HeLa cell DNA ($p<0.05$) at a concentration of 0.41 mg/ml (the IC_{50}), as shown in Figs. 5 and 6. Therefore, the results of the present study can provide important guidelines for the consumption of extracts of this plant, which is particularly common in Thailand. The cytotoxicity testing of lymphocytes and HeLa cells indicate that the leaf extract is relatively safe, without major toxicity at the cellular level.

DMSO was used to dissolve the *T. triandra* extracts for the cytotoxicity and genotoxicity analyses, and there is clearly evidence that DMSO is an unusually nontoxic organic solvent with increased use in pharmaceutical synthesis, electronics manufacturing and drug delivery. Its use is supported by over 45 years of industrial and academic experience (Gaylord Chemical Company, L.L.C.: www.gaylordchemical. com). Therefore, the results of the present study using DMSO as the solvent adequately reflect the activities of the phytochemicals present in *T. triandra* leaves.

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