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Research article

Toxicity evaluation of water extract of tissue-cultured *Taraxacum formosanum* by acute, subacute administration, and Ames test

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ABSTRACT

Background: Taraxacum species (commonly known as dandelion) used as herbal medicine have been reported to exhibit an antiproliferative effect on hepatoma cells and antitumor activity in non-small-cell lung cancer cells. Although several investigations have demonstrated the safety of *Taraxacum officinale*, the safety of tissue-cultured plants of *T. formosanum* has not been assessed so far. Therefore, the present study examines the safety of the water extract of the entire plant of tissue cultured *T. formosanum* based on acute and subacute toxicity tests in rats, as well as the Ames tests.

Results: No death or toxicity symptoms were observed in the acute and subacute tests. The results of the acute test revealed that the LD_{50} (50% of lethal dose) value of the *T. formosanum* water extract for rats exceeded 5 g/kg bw. No abnormal changes in the body weight, weekly food consumption, organ weight, or hematological, biochemical, and morphological parameters were observed in the subacute toxicity test. Thus, the no observed adverse effect level (NOAEL) of *T. formosanum* water extract was estimated to be higher than 2.0 g/kg. Finally, the results of the Ames test revealed that *T. formosanum* water extract was not genotoxic at any tested concentration to any of five *Salmonella* strains.

Conclusions: The water extract of tissue-cultured *T. formosanum* was non-toxic to rats in acute and subacute tests and exhibited no genotoxicity to five *Salmonella* strains.

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1. Introduction

Among several species of Genus *Taraxacum* (commonly known as dandelion), *Taraxacum officinale* is used as herbal medicine in Europe [1], while *Taraxacum mongolicum* is a well-known traditional medicine in China [2]. *Taraxacum formosanum* Kitamura is an endemic species in the littoral zone of the north of Taichung city in Taiwan [3] and recently has been included as an endangered species in Taiwan [4]. Among these species, *T. officinale* is the most common, and its numerous therapeutic effects have been proved, including protective effects on the liver and lung, as well as anti-virus, anti-hyperglycemic, and anti-cancer effects. *T. mongolicum* has exhibited an anticancer effect on AGS human gastric cancer cells and B162 F2 mouse melanoma cells [5,6]. *T. mongolicum* also has an anti-inflammatory effect by the regulation of the toll-like receptor 4/IκB kinase/nuclear factor-κB signal pathway [7], and the ethyl acetate fraction of *T. mongolicum* flowers has high anti-bacterial ability against Gram-negative and Gram-positive bacteria, with minimum inhibitory concentration values of 62.5 to 250 μg/mL [8]. *T. formosanum* is a Chinese medicinal herb used in Taiwan as well as mainland China. A whole-plant extract of *T. formosanum* is a common folk drink containing many functional ingredients and is traditionally used for the treatment of many diseases, including urethral infection, acute appendicitis, and lung and breast abscesses [9]. Moreover, *Taraxacum* species have been reported to have several biological activities, including anti-inflammatory and antioxidant activities, in addition to exhibiting an antiproliferative effect on hepatoma cells, and antitumor

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activity in non-small-cell lung cancer cells [10,11,12]. However, indiscriminate collection, limited geographical distribution, less frequency of self-pollination, and the value on the market of commercial crude drug, *T. formosanum* is now rarely found in its natural habitat. Therefore, propagation by tissue culture system was established (Fig. 1a-c) for its potential exploitation for commercial use [13,14]. In the present study, tissue culture-derived plants of *T. formosanum* were evaluated for its safety as food.

Many researchers have demonstrated the therapeutic benefits of dandelion, but the safety of treatments by using dandelion must be confirmed before they can be used. The toxicity of *T. officinale* is reportedly quite low as it does not contain toxic compounds or alkaloids [15]. Its LD_{50} values for mice with intraperitoneal (IP) injection of herb and root extracts of *T. officinale* are 28.8 g/kg body weight (bw) and 36.6 g/kg bw, respectively [16]. The ethanol extract of *T. officinale* that is used to treat rats and mice at doses of 10 g/kg bw with per oral administration and 4 g/kg bw with IP injection exhibits very low toxicity [15]. When *T. officinale* was administered to rabbits at doses of 3–6 g/kg bw, no acute toxicity symptom was observed during the experimental period [17]. Although several investigations have demonstrated the safety of *T. officinale*, the safety of tissue cultured *T. formosanum* has not been comprehensively explored. Therefore, this study focuses on assessing the safety of the water extract of the entire plant of tissue cultured *T. formosanum* based on acute and subacute toxicity tests in rats, as well as the Ames tests.

2. Material and methods

2.1. Chemicals

Folin–Ciocalteu reagent, sodium chloride, sodium carbonate, aluminum chloride hexahydrate, 85% orthophosphoric acid, acetonitrile, histidine, biotin, and nutrient broth were obtained from Merck (Germany). Ethanol, gallic acid, rutin, caffeic acid, 4-nitroquinoline-N-oxide, sodium azide, 9-aminoacridine, mitomycin C, and 2-aminoanthracene were purchased from Sigma Chemical Company (USA). Agar was bought from Difco (USA), and the S9 mix was purchased from Nature Opera Biotechnology Inc. (Taiwan).

2.2. Preparation of two extracts of T. formosanum

Samples of tissue culture plants were propagated by our *in vitro* propagation system [14], tissue culture plantlets were transferred to Taiwan Seed Improvement and Propagation Station, Council of Agriculture, in Xinshe, Taichung, Taiwan, and then collected after one year of growth. The commercial dandelions were obtained from two herbal medicine stores in Taipei and Taichung in Taiwan. All plant samples were dried at 37°C and identified by the method of Chiang [18]. These samples were extracted using water or ethanol. The water extract was made by extracting 1 g of *T. formosanum* powder in 100 mL of water at 90°C for 2 h. The ethanol extract was obtained by extracting 1 g of *T. formosanum* powder using 100 mL of 70% ethanol at 40°C for 2 h. The extraction solutions were filtered, and the ethanol extract was then concentrated using a rotary evaporator (EYELA, Japan) to a volume of 30 mL. Subsequently, the two extracts were lyophilized (Zirbus cavoi, Germany) and resuspended in reverse osmosis (RO) water for chemical analysis.

2.3. Total phenol and flavonoid contents

The total phenol contents of *T. formosanum* extract were measured by Folin–Ciocalteu assay [19]. Fifty microliters of each extract were mixed with 250 μL of 10% Folin–Ciocalteu reagent. After 200 μL of 7.5%, Na_2CO_3 was added, the mixture was incubated at room temperature for 10 min. The total phenol content was measured from the absorbance at 765 nm with gallic acid as the standard. The results thus obtained were expressed as mg of gallic acid equivalent per g dry

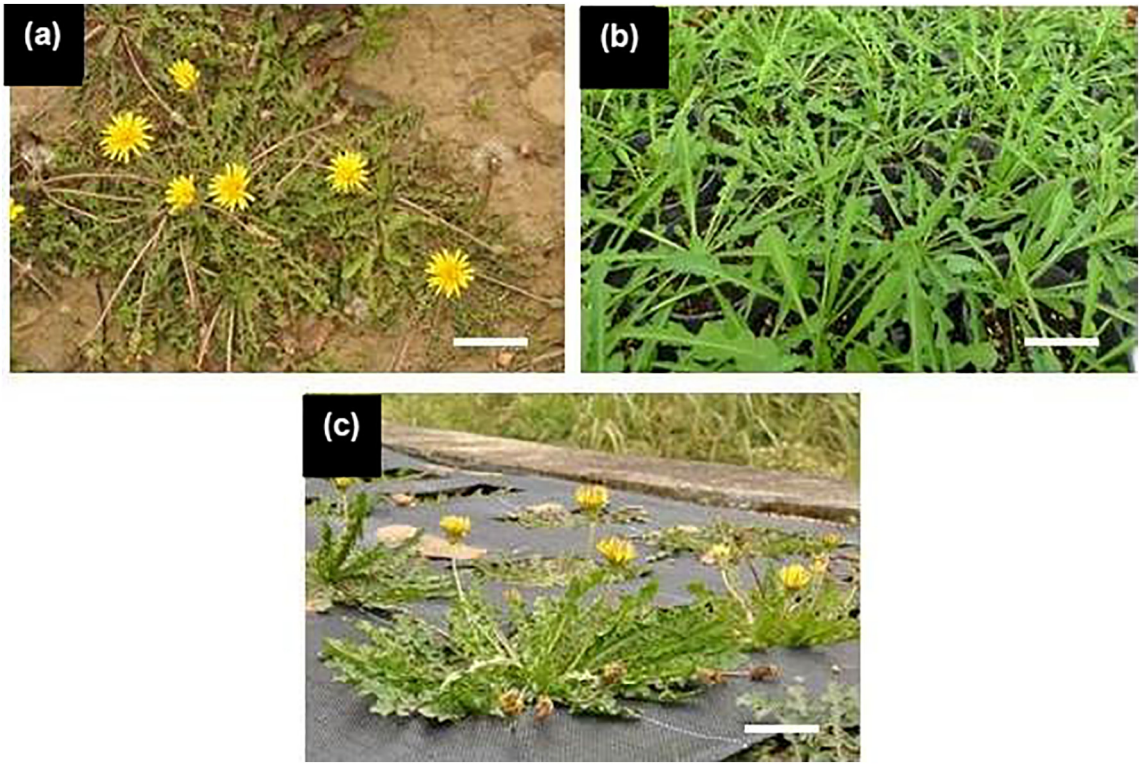

Fig. 1. (a) Wild type *Taraxacum formosanum* in Houlong Township, Miaoli County, Taiwan (Bar = 6.0 cm). (b) Potted plants in greenhouse after two months of transfer from *in vitro* conditions (Bar = 8.0 cm). (c) Tissue cultured plants in the Herbal Farm after six months of transfer from the greenhouse (Bar = 6.0 cm).

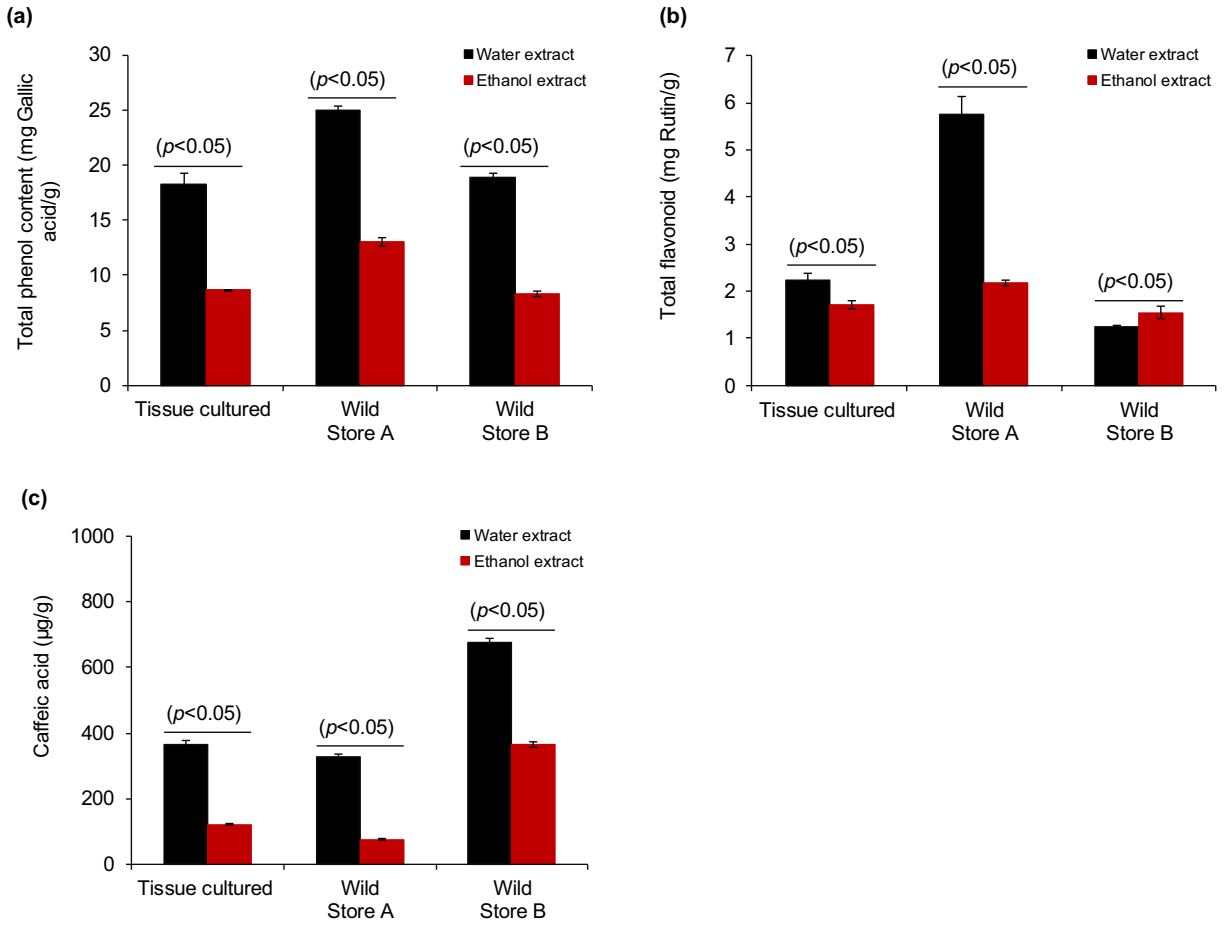

Fig. 2. Contents of (a) total phenols, (b) flavonoids and (c) caffeic acid in the water extract and ethanol extract of commercial and tissue-cultured *T. formosanum*.

weight. The method of Woisky with minor modification was used to measure the flavonoid contents of the different extracts [20]. In brief, 200 μL of extract was mixed with 200 μL of 2% $AlCl_3$ and then incubated at room temperature for 10 min. The absorbance was measured at 420 nm with rutin as a standard, and the result was expressed as mg of rutin equipment per g dry weight.

2.4. High performance liquid chromatography (HPLC) analysis of caffeic acid content

The caffeic acid content of each extract was determined by HPLC (Shimadzu, Japan) by a slightly modified version of the method of Hsieh [21]. A Purospher® STAR RP-18e column (250 mm × 4 mm, 5 μm) was used to separate samples using a gradient mobile phase that comprised 90% acetonitrile that contained 0.1% H_3PO_4 (solvent A) and 25% acetonitrile that contained 0.1% H_3PO_4 (solvent B). The volume ratio of solvent A in the mobile phase was maintained at 100% for 24 min and reduced to 20% at 30 min. The detection wavelength was 350 nm, and the flow rate was maintained at 1.0 mL/min. The column temperature was set to 35°C, and the retention time of caffeic acid was 12 min.

2.5. Animals

Male and Female Sprague–Dawley rats, aged five weeks, were purchased from BioLASCO Company in Taipei City, Taiwan. The animals were maintained in a standard environment (23–27°C; 50–

Table 1
Results of hematological parameters and serum biochemistry of rats after rats were fed with water extract of tissue cultured *T. formosanum* by oral gavage in acute oral toxicity test.

Dose (mg/kg)	WBC (10^3/μL)	RBC (10^3/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/DL)	PLT (10^3/μL)	LYM (10^3/μL)	AST (U/L)	ALT (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
Male													
Control	4.9 ± 1.2[a]	7.2 ± 0.6	13.9 ± 1.2	44.1 ± 3.3	61.3 ± 1.3	19.3 ± 0.3	31.5 ± 0.6	1144.8 ± 76.6	3.7 ± 1.0	83.60 ± 12.62	36.20 ± 5.22	13.80 ± 4.97	0.68 ± 0.04
5.0	5.9 ± 0.7	7.6 ± 0.4	14.6 ± 1.0	46.1 ± 2.0	60.6 ± 0.9	19.2 ± 0.9	31.7 ± 1.5	1085.2 ± 177.0	4.7 ± 0.5	79.40 ± 5.86	34.20 ± 2.77	13.80 ± 2.28	0.66 ± 0.05
Female													
Control	4.9 ± 0.3	7.4 ± 0.2	14.1 ± 0.2	43.7 ± 0.8	59.4 ± 1.1	19.1 ± 0.5	32.2 ± 0.6	1214.2 ± 230.9	3.9 ± 0.4	77.00 ± 4.74	27.60 ± 3.85	19.60 ± 4.83	0.83 ± 0.10
5.0	5.0 ± 0.9	7.5 ± 0.3	14.2 ± 0.1	43.9 ± 0.9	58.8 ± 1.2	19.0 ± 0.6	32.3 ± 0.5	1285.5 ± 180.7	3.8 ± 0.4	80.25 ± 5.56	28.75 ± 4.99	22.00 ± 3.46	0.76 ± 0.05

WBC: white blood cell, RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen.
Data were expressed mean ± SD (n = 5).
[a] Significant difference between the control and treated group.

Table 2
Results of hematological parameters of rats after rats were fed with water extract of tissue cultured *T. formosanum* by oral gavage in 28 day oral toxicity test.

Dose (mg/kg)	WBC (10^3/μL)	RBC (10^3/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/DL)	PLT (10^3/μL)
Male								
Control	5.1 ± 2.1	8.3 ± 0.2	15.9 ± 0.5	50.3 ± 1.7	60.6 ± 1.2	19.2 ± 0.7	31.7 ± 1.0	1151.3 ± 201.7
0.5	3.8 ± 1.2	8.2 ± 0.3	15.4 ± 0.3	48.8 ± 1.1	59.3 ± 2.1	18.8 ± 0.9	31.6 ± 0.5	1058.2 ± 76.2
1.0	5.9 ± 1.4	8.2 ± 0.3	15.5 ± 0.4	48.8 ± 1.8	59.7 ± 1.8	19.0 ± 0.8	31.8 ± 0.8	1090.6 ± 210.7
2.0	3.7 ± 0.5	8.5 ± 0.6	15.5 ± 0.4	50.0 ± 2.2	59.1 ± 2.2	18.3 ± 1.0	31.1 ± 1.0	1200.0 ± 107.5
Female								
Control	6.4 ± 1.7	7.9 ± 0.4	15.0 ± 0.5	46.8 ± 2.3	58.9 ± 1.3	18.9 ± 0.6	32.0 ± 0.6	1229.4 ± 171.2
0.5	3.5 ± 0.9[a]	7.9 ± 0.2	14.7 ± 0.5	45.8 ± 1.5	58.3 ± 1.1	18.7 ± 0.4	32.1 ± 0.4	1198.6 ± 224.8
1.0	4.3 ± 2.4	7.8 ± 0.4	14.8 ± 0.7	46.7 ± 2.5	59.8 ± 0.9	19.0 ± 0.2	31.8 ± 0.4	1114.6 ± 353.4
2.0	3.7 ± 1.9[a]	7.5 ± 0.2	14.4 ± 0.4	43.8 ± 0.7[a]	58.6 ± 1.3	19.2 ± 0.7	31.1 ± 1.0	1209.6 ± 134.0

WBC: white blood cell, RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelet.
Data were expressed mean ± SD (n = 5).
[a] Significant difference between the control group and treated group.

70% air humidity; 12/12 h dark/light cycle). All animals were supplied with standard pellet diet (LabDiet, Taiwan) and water *ad libitum*. All of the processes in the animal experiment were carried out according to the institutional guidelines that were approved by the Animal Care and Use Committee of National Chung Hsing University (Taichung, Taiwan).

2.6. Acute toxicity

Ten male rats or ten female rats were separated into two groups, the control group, and the experimental group. The animals fasted overnight but supplied with water *ad libitum*. The control group (n = 5/group) was orally administrated RO water, and the experimental group (n = 5/group) was gavaged 5 g/kg of *T. formosanum* water extract on Day 0. Changes in the weight and clinical symptoms of animals were observed daily until Day 14. At the end of the treatment, all rats were sacrificed, and their organs and blood were collected to evaluate pathological or biochemical changes.

2.7. Subacute toxicity

The rats were divided into four groups, and each group consisted of five male and five female rats. The control group was orally administered RO water, and the experimental groups were treated with *T. formosanum* water extract at doses of 0.5, 1.0, and 2.0 g/kg bw/day for 28 d continuously. Bodyweight and food consumption were recorded every week, and signs of toxicity were observed throughout the experiment. Neither water nor food was supplied to rats for 12–16 h on Day 28 after *T. formosanum* water extract was administered, and plastic trays were placed under the cage to collect urine for analysis. Water and food were provided *ad libitum* after the urine was removed and collected the day after the disks were installed. On Day 29, all rats fasted overnight and were anesthetized on Day 30 to collect blood from the aorta abdominals using K_3 ethylenediamine tetraacetic acid syringes (Vacutainer, USA), serum separator tubes (Vacationer, USA), and 3.8% buffered sodium citrate tubes (Becton Dickson, USA). Organs of the rats were collected, and their macroscopic and microscopic features were observed.

2.8. Hematological and blood serum biochemical analysis

The hematological analysis was performed using an automatic hematological analyzer (Sysmex K-450, Japan). The whole blood analysis included a white blood cell (WBC) count, a red blood cell (RBC) count, hemoglobin (HGB) level, mean corpuscular hemoglobin (MCH) level, mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count. Blood serum was collected by being centrifuged at 3000 rpm for 10 min, and used to measure the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, total cholesterol, triglycerides, alkaline phosphatase (ALP), total protein, albumin, total bilirubin, amylase, creatine kinase, magnesium (Mg^{2+}), calcium (Ca^{2+}), phosphorus (P^{3-}), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), gamma-glutamyl transpeptidase (GGT), and fibronectin (Fbg), as well as the activated partial thromboplastin time (APTT), the prothrombin time (PT) and glucose level.

2.9. Urinalysis

Rats were starved overnight without water on Day 0 and Day 28, and their urine was collected on each of the following days. The properties of the urine, including the pH value, concentrations of protein, bilirubin,

Table 3
Results of urinary analyses and urinary sediments in rats after rats were fed with *T. formosanum* water extract in the 28-days oral toxicity test.

Dose (mg/kg)	Volume (mL)	Specificity gravity	pH	Protein (mg/dL)	Urobilinogen (mg/dL)	Urinary sediments (10^3)			
						RBC	WBC	Epithelial cells	Cast
Male									
Control	4.4 ± 0.8	1.0 ± 0.0	7.2 ± 0.4	65.0 ± 22.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.1	0.0 ± 0.0
0.5	5.2 ± 1.9	1.0 ± 0.0	6.7 ± 0.4	55.0 ± 27.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.1	0.0 ± 0.0
1.0	6.4 ± 2.5	1.0 ± 0.0	7.2 ± 0.4	55.0 ± 27.4	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
2.0	6.7 ± 3.3	1.0 ± 0.0	7.4 ± 0.5	45.0 ± 27.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Female									
Control	2.7 ± 0.6	1.0 ± 0.0	6.5 ± 0.4	75.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.5	2.2 ± 1.0	1.0 ± 0.0	6.1 ± 0.2	45.0 ± 27.4[a]	0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 2.2	0.0 ± 0.0
1.0	2.8 ± 1.2	1.0 ± 0.0	6.3 ± 0.3	80.0 ± 44.7	1.2 ± 1.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2.0	3.6 ± 0.8	1.0 ± 0.0	6.3 ± 0.4	35.0 ± 22.4[a]	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

RBC: red blood cell. WBC: white blood cell.
Data were expressed mean ± SD (n = 5).
[a] Significant difference between the control group and treated group.

urobilirubin, ketones, nitrite, and occult blood, as well as the specific gravity, were analyzed using a urine test strip and a urine analyzer (Miles Inc., USA). A hemocytometer was used to measure the WBC, RBC, epithelial cell, cast, and crystal contents in the urine.

2.10. Organ weights and morphologies

The weights of the thymus, heart, liver, spleen, kidney, adrenal gland, brain, and reproductive organs (testicle or ovary) were obtained after the animals were sacrificed. Organ weights are expressed in relative terms (organ weight (g)/body weight (g) × 100%). All organs were preserved in 10% formalin solution. Tissue slices were obtained using the method of Lison and stained with hematoxylin/eosin. The microscopic features of the organs were observed using an optical microscope (Olympus, Japan) and photographed.

2.11. Mutagenicity assessment

The five bacterial strains - *Salmonella* TA98, TA100, TA102, TA1535, and TA1537 – were used in mutagenic tests, following the Health Food Safety Assessment guidelines that are set by the Department of Health of the Executive Yuan of the Republic of China. Before the Ames test, whether the *T. formosanum* water extract was at all toxic to the five bacterial strains had to be determined. Bacterial solutions were diluted to 10^6 and 10^7 CFU/mL using sterilized distilled water. One hundred microliters of each diluted bacterial solution was mixed with 100 μL of different concentrations (5.0, 2.5, and 1.25 mg/mL) of *T. formosanum* water extract. The mixture of bacterial solution and *T. formosanum* water extract was added to liquid soft agar, placed on the nutrient agar plates, and incubated at 37°C for 24 h. The bacterial toxicity test was performed in triplicate on test samples of each concentration.

The reference mutagens without enzyme S9 as the positive control were prepared as follows: 2.5 μg/plate 4-nitroquinoline-N-oxide for TA98, 5 μg/plate sodium azide for TA100 and TA1535, 50 μg/plate 9-aminoacridine for TA1537, and 0.5 μg/plate mitomycin C for TA102. Various concentrations (5.0, 2.5, 1.25, 0.625, and 0.3125 mg/mL) of *T. formosanum* water extract were mixed with 100 μL of bacterial solution and 200 μL of 0.5 mM histidine/biotin or 100 μL of enzyme S9. Each solution was then mixed with 2 mL of soft agar and placed on a minimal glucose agar plate. The plates were incubated at 37°C for 48 h, and the colonies were counted, and their numbers recorded.

2.12. Statistical analysis

Data are expressed as mean ± standard deviation. The Statistics Analysis System was used to compare the animal toxicity test results for the control group, and the treated group and the mutagenicity were obtained from the results of the Ames test by performing the student t-test. A probability (p-value) of under 5% ($p < 0.05$) was regarded as indicating a significant difference.

3. Results

3.1. Analysis of antioxidant compound and caffeic acid contents

Water and 70% ethanol was used as extraction solvents to obtain high amounts of antioxidant compounds from *T. formosanum*. Tissue-cultured *T. formosanum* and wild *T. formosanum* from two traditional Chinese medicine stores were compared. The results reveal that the total phenol, total flavonoid, and caffeic acid contents of the water extract of *T. formosanum* were significantly higher than those of the 70% ethanol extract of *T. formosanum* from both the tissue-cultured *T. formosanum* and the wild *T. formosanum* samples (Fig. 2A-C). Therefore, water was utilized as the extraction solvent in the following experiment.

3.2. Acute toxicity

The acute toxicity test was performed to determine the LD_{50} dosage in rats. Male and female rats were fed 5 g/kg bw of *T. formosanum* water extract by oral gavage. No toxicity or death was observed. The hematological parameters and serum biochemistry of the rats were analyzed. The results in Table 1 indicate that no significant difference between the hematological parameters and serum biochemistry of the

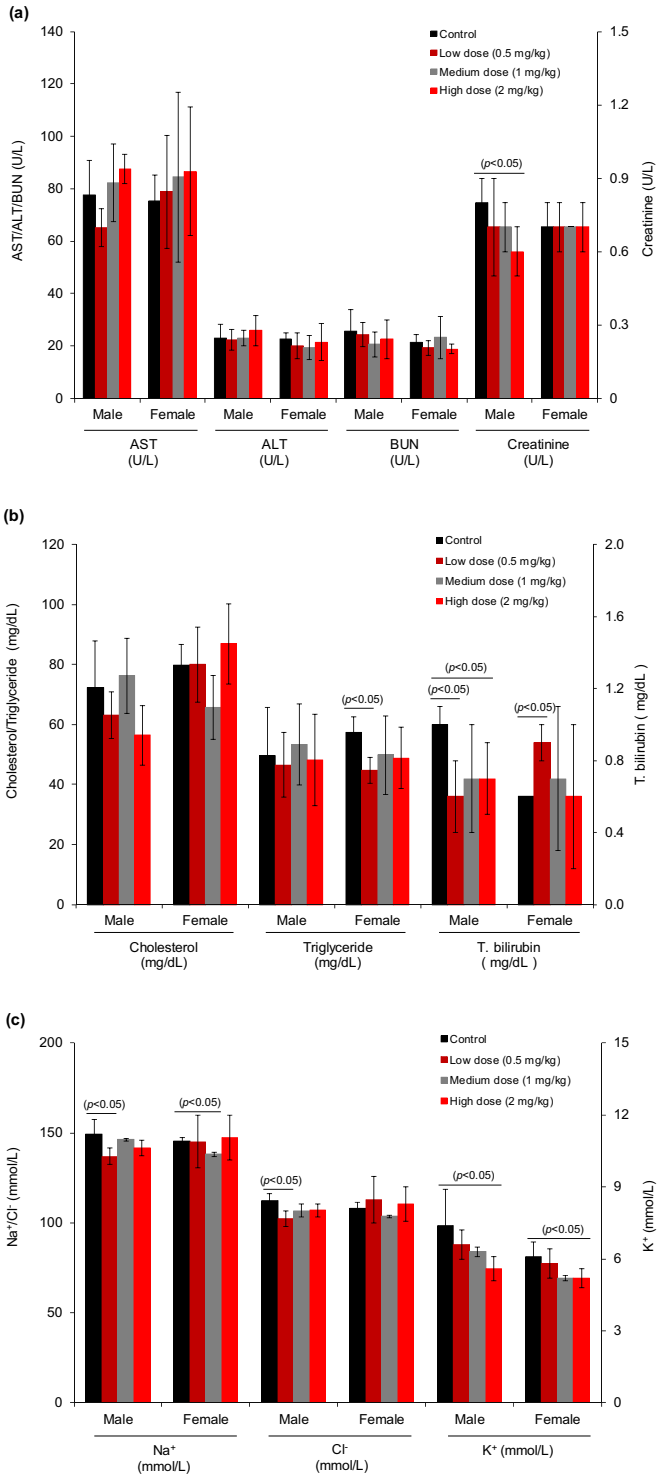

Fig. 3. Results of serum biochemistry of rats fed with water extract of tissue-cultured *T. formosanum* by oral gavage in the 28-day oral toxicity test. (a) Liver and kidney function. (b) Liver function and blood lipids contents. (c) Ion concentration.

Table 4
Bacterial toxicity test of water extract of tissue cultured *T. formosanum*.

Tester strains	Bacterial concentration	*T. formosanum* water extract (mg/plate)/No. of revertanted (colony, CFU/plate)			
		0	1.25	2.5	5
TA98	10^{-6}	241.7 ± 6.0[a]	239.7 ± 5.5	233.3 ± 8.1	237.0 ± 5.0
	10^{-7}	22.3 ± 5.7	23.3 ± 3.5	30.0 ± 7.9	17.0 ± 6.0
TA100	10^{-6}	127.3 ± 5.5	132.0 ± 1.0	115.7 ± 8.1	118.0 ± 7.0
	10^{-7}	13.7 ± 2.1	12.0 ± 1.0	14.3 ± 1.5	14.0 ± 1.0
TA102	10^{-6}	133.7 ± 3.2	138.0 ± 4.6	135.7 ± 3.8	138.0 ± 7.9
	10^{-7}	14.0 ± 5.0	13.3 ± 0.6	15.7 ± 4.5	12.3 ± 0.6
TA1535	10^{-6}	149.3 ± 3.5	142.0 ± 5.6	145.0 ± 8.1	147.7 ± 5.5
	10^{-7}	17.0 ± 4.4	12.3 ± 1.2	14.0 ± 2.7	12.7 ± 4.0
TA1537	10^{-6}	146.0 ± 6.0	147.7 ± 2.5	153.3 ± 2.9	146.0 ± 1.0
	10^{-7}	14.0 ± 2.7	14.7 ± 2.1	13.0 ± 0.7	13.3 ± 1.5

Data were expressed as mean ± SD (n = 3).
[a] Significant between the control groups and treated groups.

control group and those of the treatment group. The weekly body weight, relative organ weights, macroscopic organ morphologies, and histopathologies also did not differ significantly between the treatment group and the control group (data not shown). Therefore, based on the hematological parameters and serum biochemistry, the LD_{50} of the *T. formosanum* water extract was determined to be greater than 5 g/kg bw for rats.

3.3. Subacute toxicity

For the subacute toxicity test, rats were fed 0.5, 1.0, and 2.0 g/kg bw of *T. formosanum* water extract by oral gavage, and the hematological parameters, serum biochemistry, urine, and urinary sediments were analyzed. No toxicity or deaths was observed during the 28 d after rats were treated with *T. formosanum* water extract. Treatments with doses of 0.5, 1.0, and 2.0 g/kg bw of *T. formosanum* water extract did not affect weekly body weight or food consumption (data not shown). Treatment with *T. formosanum* water extract did not significantly alter the hematological parameters, except in that the WBC of the female rats that were treated with 0.5 g/kg bw and 2.0 g/kg bw and the hematocrit (HCT) of the female rats that were treated with 2.0 g/kg bw were significantly lower than those of the control group (Table 2). However, the HCT and WBC values for the rats were all within the reference range [22]. The results of the serum biochemical analysis in Table 3 demonstrate that most of the values of treatment groups did not differ significantly from those of the control groups, with the exceptions of creatinine, total bilirubin (T. bilirubin), cholesterol, triglyceride, Na^+, K^+, and Cl^- (Fig. 3a-c). Treatment with *T. formosanum* water extract did not affect the urine or urinary sediments, except in that the protein content of the urine in the female rat groups that were treated with 0.5 g/kg bw and 2.0 g/kg bw was lower than that of the female control group (Table 3). Treatment with *T. formosanum* water extract did not affect relative organ weight, the texture or color of the organs, or the results of the microscopic histopathological analysis (data not shown).

3.4. Ames test

To estimate bacterial toxicity, 1.25, 2.5, and 5.0 mg/plate *T. formosanum* water extracts were used. The results in Table 4 indicate that the 5 mg/plate *T. formosanum* water extract exhibited no toxicity toward the five strains of *Salmonella typhimurium* - TA98, TA100, TA102, TA1535 and TA1537, as the colony counts of bacteria that were exposed to 1.25, 2.5, and 5 mg/plate of *T. formosanum* water extract did not differ significantly from that of the control group (Table 4). Based on the results of the bacterial toxicity test, five concentrations of *T. formosanum* water extract from 0.3125 to 5.0 mg/plate were utilized in the Ames test. According to Table 5, treatment with various doses of *T. formosanum* water extract (0.3125 to 5 mg/plate) did not affect bacterial colonies, revealing that the *T. formosanum* water extract exhibited no mutagenicity toward any of the five strains.

Table 5
Ames test of tissue cultured *T. formosanum*.

Tester strains	Liver microsomal enzyme	*T. formosanum* water extract (mg/plate)/No. of revertanted (colony, CFU/plate)						
		NC	PC	0.3125	0.625	1.25	2.5	5
TA98	-S9	26.7 ± 6.0	97.7 ± 9.3[a]	26.0 ± 8.5	31.0 ± 5.3	20.3 ± 5.5	20.7 ± 1.2	25.0 ± 3.5
	+S9	24.7 ± 0.6	1741.7 ± 30.0[a]	23.3 ± 2.3	26.0 ± 2.0	24.7 ± 2.1	27.3 ± 2.1	23.3 ± 1.5
TA100	-S9	129.3 ± 6.8	1507.0 ± 34.1[a]	126.3 ± 3.8	130.0 ± 7.0	142.0 ± 6.6	146.3 ± 9.0	144.7 ± 8.4
	+S9	108.7 ± 1.5	2384.0 ± 63.7[a]	117.7 ± 6.5	120.7 ± 8.0	119.3 ± 8.0	117.3 ± 8.6	112.0 ± 7.0
TA102	-S9	289.0 ± 7.2	2128.0 ± 122.3[a]	278.0 ± 6.6	282.3 ± 9.9	280.7 ± 6.7	293.3 ± 6.7	288.0 ± 9.1
	+S9	397.3 ± 5.7	1983.3 ± 22.5[a]	395.0 ± 5.6	391.7 ± 6.5	393.7 ± 6.1	396.3 ± 6.7	395.7 ± 7.6
TA1535	-S9	9.0 ± 1.7	1491.0 ± 170.0[a]	12.7 ± 2.5	11.0 ± 3.6	15.3 ± 4.0	10.7 ± 4.0	13.3 ± 4.7
	+S9	14.3 ± 2.5	276.0 ± 29.0[a]	15.3 ± 2.3	16.0 ± 3.6	16.3 ± 5.1	17.3 ± 8.0	17.7 ± 6.5
TA1537	-S9	10.7 ± 0.6	2146.0 ± 90.0[a]	14.0 ± 1.0	10.3 ± 1.5	16.7 ± 0.6	12.7 ± 3.6	15.7 ± 3.5
	+S9	11.0 ± 1.0	408.0 ± 39.0[a]	8.0 ± 2.0	7.0 ± 2.6	11.3 ± 1.5	7.3 ± 2.5	8.0 ± 2.7

Negative control (NC): sterile water.
Positive control (PC): TA98 was treated to 2.5 μg/plate 4-nitroquinoline-N-oxide, TA100 and TA1535 were treated to 5 μg/plate sodium azide, TA102 was treated to 0.5 μg/plate mitomycin C, TA1537 was treated to 50 μg/plate 9-aminoacridine in condition of non S9 addition;
All *Salmonella typhimurium* strains were added to 2-aminoanthracene in condition of S9 addition.
Data are expressed as mean ± SD (n = 3).
[a] Significant between the control groups and treated groups.

4. Discussion

A whole-plant extract of Chinese medicinal herb *T. formosanum* is traditionally used as folk drink and for the treatment of several diseases including urethral infection, acute appendicitis, and lung and breast abscesses in Taiwan and mainland China [9] and so far there is no report of any toxicity of crude drug derived from wild plants. Hence, the present study was confined to tissue culture derived plants alone. Due to endemic and endangered nature of *T. formosanum* species (reference), availability of enough wild plant material is a serious constraint. This can be evident from only a few research reports on *T. formosanum* [12]. Tissue culture plants developed by vegetative parts of wild plants in general are considered as true to type (clones) [23,24,25] hence authors did not anticipate any difference between tissue culture and wild plants. However, since there is no report so far on the toxicity evaluation of tissue culture derived material of any medicinal plant species, authors considered to carry out the present study as an important piece of research.

In the acute toxicity test, rats that were treated with 5 g/kg bw of *T. formosanum* water extract experienced no toxicity. Therefore, the LD_{50} values of the *T. formosanum* water extract were estimated to exceed 5 g/kg bw. Since an LD_{50} value within 1–5 g/kg bw is regarded as a very low toxicity, the *T. formosanum* water extract can be classified as safe [26]. In the 28 d subacute oral toxicity test, most of the results of the hematological and blood serum biochemical analyses of the treatment groups did not differ significantly from those of the control group, except for those concerning the value of creatinine, cholesterol, triglyceride, Na^+, and Cl^-. However, all such values were within the normal reference ranges [22]. Creatinine is an indicator of renal function, and is metabolized from muscle creatine and excreted by the kidneys [27]. Therefore, concerning the impact of *T. formosanum* water extract on renal function, the level of creatinine in male rats that had been treated with 2.0 g/kg bw of *T. formosanum* water extract was significantly lower than that of the male control group. However, the female rats did not exhibit this effect. The protein contents of the urine of the 0.5 g/kg and 2 g/kg dose female rats were significantly lower than that of the female control group, but not in a dose-dependent manner (Table 3). No histopathological damage to the kidneys of the treated rats was observed, indicating that treatment with *T. formosanum* did not affect renal function.

In circulating blood, bilirubin normally binds to albumin because it is insoluble in water, and albumin-bound bilirubin can be excreted into the bile through the liver or the kidney. Therefore, bilirubin is an indicator of liver and renal functions [28]. In this study, the levels of T. bilirubin in male rats that were treated with 0.5 and 2.0 g/kg bw of *T. formosanum* water extract were lower than that of the control group. However, the level of T. bilirubin in the female group that was treated with 0.5 g/kg bw of *T. formosanum* water extract exceeded that of the control group. However, the male rats yielded no similar finding, and all levels of T. bilirubin were within the normal reference range (Fig. 3a–c) [22]. Therefore, treatment with *T. formosanum* water extract did not affect the T. bilirubin level in both male and female rats.

Reducing serum triglyceride (TG) or cholesterol level is considered to improve health. The level of TG in female rats that were treated with 0.5 g/kg bw of *T. formosanum* water extract and the level of cholesterol in female rats that were treated with 1.0 g/kg bw of *T. formosanum* water extract was lower than those of the control group (Fig. 3a–c). These results indicate that *T. formosanum* water extract may reduce serum cholesterol and *T. formosanum* levels, but more research is required to prove this claim. Serum electrolytes have an important role in keeping fluids in balance in the body. In this study, *T. formosanum* water extract reduced the serum potassium content in a dose-dependent manner, perhaps because dandelion is a diuretic. Moreover, urine volumes of the treatment groups increased with the dose of *T. formosanum* water extract (Table 3). Based on these results, *T. formosanum* water extract exhibits a diuretic activity. Several studies have proved that extracts from dandelion roots (*Taraxaci radix* and *T. officinale*) or dandelion herb (*Taraxaci folium*) had diuretic activity since urine volume increased after administration of these extracts. The high potassium content in dandelion is considered responsible for the diuretic activity of dandelion [16,29].

Histopathology was utilized to evaluate the toxicity of tested drugs [30]. In this study, a histopathological examination of the liver in the subacute oral toxicity test revealed that *T. formosanum* water extract had no toxicological effects. Based on the results of this study, the NOAEL of *T. formosanum* water extract was 2 g/kg bw for rats, and the acceptable daily intake for humans is equivalent to 20 mg/kg/day.

5. Conclusions

The water extract of *T. formosanum* contained total phenol, total flavonoid, and caffeic acid than the ethanol extract of *T. formosanum*. The water extract is more easily prepared than the ethanol extract because no organic solvent has to be removed before it is used. Therefore, water is a good extracting solvent for *T. formosanum*. In the animal toxicity test, the parameters of treated rats, including body and organ weights, serum biochemistry, and histopathology, did not significantly differ from those of the control group. No organ pathology or toxicity symptoms were observed in rats that had been administered *T. formosanum* water extract. The LD_{50} and NOAEL of *T. formosanum* for rats are 5 g/kg/bw and 2 g/kg/bw, respectively. In the Ames test, none of the five test strains exhibited mutagenicity when exposed to *T. formosanum* water extract with a concentration of 5 mg/plate, indicating that *T. formosanum* exhibits no genotoxicity. Based on the results in this study, *T. formosanum* is a safe herb, but the functional effects of *T. formosanum* have yet to be investigated.

Ethical approval

Authors declare all of the processes in the animal experiment were carried out according to the institutional guidelines that were approved by the Animal Care and Use Committee of National Chung Hsing University, Taiwan.

Conflict of interest

The authors declare that there are no conflicts of interest among them.

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