RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.096

ScienceAsia 49 (2023): **873–879**

Antioxidant, cytotoxic activities, and phytochemical compositions of the Thai traditional medicine Trikanrapit remedy

Anusara Sihanat[a], Arunporn Itharat[b,c], Patsorn Worawattananutai[d], Kriyapa Lairungruang[e], Nattiya Chaichamnong[a], Nobpawan Watcharaput[a], Pawarisa Noppakao[a], Rodsarin Yamprasert[a,*]

[a] Division of Applied Thai Traditional Medicine, Faculty of Public Health, Naresuan University, Phitsanulok 65000 Thailand

[b] Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120 Thailand

[c] Center of Excellence in Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120 Thailand

[d] Faculty of Allied Health Sciences, Burapha University, Chonburi 20131 Thailand

[e] Abhaibhubejhr College of Thai Traditional Medicine Prachinburi, Faculty of Public Health and Allied Health Sciences, Praboromarajchanok Institute, Ministry of Public Health, Prachin Buri 25230 Thailand

[*] Corresponding author, e-mail: rodsariny@nu.ac.th

Received 23 May 2023, Accepted 23 Sep 2023
Available online 29 Dec 2023

ABSTRACT: Trikanrapit (red basil roots, galangal rhizome, and fingerroot) is a traditional Thai herbal remedy used to cure body impairment, nourish blood and sexual desire, and as a carminative drug. This study aimed to investigate the antioxidant activity via 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization (ABTS), ferric ion reducing antioxidant power (FRAP) assay and cytotoxic activity via a sulforhodamine B assay. The chemical constituents of the extracts were analyzed using gas chromatography-mass spectrometry (GC-MS). The results indicate that the ethanolic extract of Trikanrapit remedy showed good antioxidant activity, and the root extract of *Ocimum tenuiflorum*, one of the three herb constituents, exhibited strong antioxidant activity on the DPPH and $ABTS^{\cdot+}$ assays. The Trikanrapit remedy extract showed moderate cytotoxicity against LS174T colorectal adenocarcinoma cells and human lung adenocarcinoma A549 cells, with 50% maximal inhibitory concentration (IC_{50}) values of 52.04 and 46.85 µg/ml, respectively. While the root extract of *O. tenuiflorum* showed high cytotoxicity activities on both the LS174T and A549 cell lines, with IC_{50} values of 31.51 and 29.60 µg/ml, respectively. Moreover, Trikanrapit extract contains approximately 47 different compounds. In addition, the data discussed in this study revealed cytotoxic and antioxidant properties, which correspond to the traditional Thai theory of using herbal recipes to treat various diseases.

KEYWORDS: Thai traditional medicine, Trikanrapit remedy, cytotoxic activity, antioxidant activity, phytochemical composition

INTRODUCTION

Cancer is a non-communicable disease and the leading cause of chronic disease-related death worldwide with around 10 million people dying from cancer every year [1]. In 2020, the International Agency for Research on Cancer reported that female breast cancer and male lung cancer were the most commonly diagnosed cancers [2]. Lung cancer is the leading cause of cancer-related deaths, with an estimated 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%). Global cancer cases are expected to increase to 28.4 million by 2040, a 47% increase from 2020 [2]. In Thailand, the Office of Policy and Strategy of the Ministry of Public Health reported that cancer was the most prevalent cause of death in the Thai population between 1994 and 2021. Moreover, 83,795 people have died from cancer in 2021 [3]. This deadly phenomenon tends to increase gradually compared to other diseases. The dissemination and provision of cancer care efforts are focused on cancer prevention measures critical for global cancer control.

Currently various cancer treatment methods provide more effective forms of treatment for relieving patients' ailments. Medical procedures that address many issues involving cancer include surgery, radiotherapy, systemic therapy, chemotherapy, hormone antagonists, immunotherapy, genetic treatments. However, the treatment process still has limitations that are problematic for patient cure, such as the severity of cancer, type of cancer, access to appropriate treatment, cost, drug resistance, and side effects. Therefore, alternative medicines or herbal medicine therapies have begun to play a role in cancer treatment. Many anti-cancer drugs have been discovered because of studies on the properties of potent herbal chemicals. Several studies have demonstrated the effectiveness of plant extracts and phytochemicals against various cancers [4, 5].

The Trikanrapit (TKP) remedy is one of the herbal remedies in the official Thai traditional medicine textbook named "Paet-Saat-Song-Kroh (Thai word)" from the Ayuraved-Wittayarai Foundation and Thai traditional pharmacy scripture. The TKP consists of three herbs roots: *Alpinia galanga* (L.) Wild (AG), *Boesenbergia rotunda* (Linn.) Mansf (BR), and *Ocimum tenuiflorum* L (OT) in an equal ratio (1:1:1). TKP remedied are used to cure the impairment of elements in the body, nourish blood and sexual desire, and take carminative drugs. AG or galangal rhizome is a plant in the ginger family that is native to Southeast Asia. It is commonly used in traditional medicine for its anticancer, anti-inflammatory, analgesic, and antibacterial properties [6]. BR commonly known as fingerroot is a perennial herb of the Zingiberaceae family with anti-cancer, anti-leptospiral, anti-inflammatory, antioxidant, antiulcer, and anti-herpes viral activities [7]. OT, commonly known as Holy Basil or Tulsi is an important medicinal plant in the various traditional and folk systems of medicine in Southeast Asia. Scientific studies that have shown possess anti-inflammatory, analgesic, antipyretic, antidiabetic, hepatoprotective, hypolipidemic, antistress, and immunomodulatory activities [8].

However, scientific evidence supporting TKP remedy is limited, and there is a need for a better understanding of more research to build a solid scientific data-base of evidence on traditional medicine practices. The aim of the present study is to investigate the potential cytotoxic activity of TKP against colon adenocarcinoma cancer cells (LS174T), human lung adenocarcinoma cells (A549) and normal human lung myofibroblast Medical Research Council cell strain 5 (MRC-5). Additionally, the antioxidant activity and phytochemical composition of the ethanol in the remedy were evaluated. This study contributes to the growing body of scientific evidence on the potential therapeutic benefits of traditional medicine practices and highlights their potential therapeutic benefits.

MATERIALS AND METHODS

Plant materials

The BR rhizome and OT root were collected from Phitsanulok Province, and the AG rhizome was collected from Phetchabun Province. Species identification and Verification Numbers 05788, 05789, and 05790, respectively, were verified by the Herbarium in the Department of Biology, Faculty of Science, Naresuan University, Thailand. This study was approved by the Biosafety Committee of Thammasat University, Thailand under Biosafety Level 1 (Number 083/2021).

Extract preparation

The roots of OT were cleaned and dried in a hot air oven at 40 °C and the rhizomes of both the BR and AG were cleaned, autoclaved, and dried in a hot air oven at 40 °C. The dried AG, BR, and OT samples in a ratio of 1:1:1 were mechanically powdered into coarse powders and extracted by Soxhlet extraction with 95% ethanol for 24 h. The extract was then concentrated under reduced pressure using a rotary evaporator, and the yield percentage was calculated. The 95% ethanol crude extracts were stored at −20 °C until required for testing.

Cell culture

Colon adenocarcinoma cells (LS174T; no. CL-188), human lung adenocarcinoma cells (A549; no. CCL-185), and a normal human lung myofibroblast cell line (MRC-5; no. CCL-171) were obtained from the American Type Culture Collection (Manassas, VA, USA). The LS174T and MRC-5 cells were cultured in Minimum Essential Medium (MEM) and then supplemented with 10% heated fetal bovine serum (FBS), 50 IU/ml penicillin, and 50 µg/ml streptomycin. Then the A549 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO/BRL Life Technologies, USA) with 10% FBS (Sigma-Aldrich, St. Louis, MO, USA). The LS174T, A549, and MRC-5 cells were seeded in 96-well plates with an initial concentration of 3×10^3, 1×10^4, and 5×10^4 cells/well, respectively, in 100 µl medium. The cells were maintained at 37 °C in 75% relative humidified atmosphere of 5% CO_2 for 24 h. The cells were treated with various concentrations of the extracts using 1, 10, 50 or 100 µg/ml in the completed medium for 72 h. The medium was replaced with 200 µl the new media, followed by the cells being cultured for a further 72 h; then, after incubation, the viability of all the cells was measured.

Cytotoxic activity

A sulforhodamine B (SRB) assay was performed to measure cell viability [9]. The viable cells were fixed with 100 µl of 40% (w/v) trichloroacetic acid at 4 °C for 1 h and then stained with 50 µl of 0.4% (w/v) SRB solution in 1% acetic acid for 30 min. After staining, the color was dissolved in a 10 mM Trisma base solution. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The absorbance (Abs.) at 492 nm was measured using a microplate reader (Bio-Tek Instruments, Winooski, Vermont, USA). The percentage of inhibition was calculated using the following formula:

$$\%\,\text{Inhibition} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Then 50%-maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, USA).

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity was determined using DPPH according to the modified method of Yamasaki et al [10]. This was followed by 180 µl of 6×10^{-5} M DPPH solution in absolute ethanol being mixed with 20 µl of different concentrations of the extract, then incubated for 30 min in the dark at a room temperature of 20 to 25 °C.

Following incubation for 30 min, absorbance was measured at 520 nm using a microplate reader. Butylated hydroxytoluene (BHT) was used as the positive control because of its antioxidant properties. Initially, DPPH 50%-maximal response effective concentration (EC_{50}), a parameter widely used to measure antioxidant activity, was determined, and defined as the concentration required to reduce DPPH activity by 50%. The antioxidant scavenging activity was calculated as an inhibition percentage using the formula above and expressed as IC_{50} (µg/ml).

2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay

$ABTS^{\cdot+}$ radical scavenging assay was conducted according to the method described by Re [11]. The ABTS assay involves two steps: the preparation of the ABTS radical solution and the discoloration assay. The $ABTS^{\cdot+}$ radical solution was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate. The reaction mixture was allowed to stand in the dark at room temperature of 20 to 25 °C for 12–16 h to complete radical generation. Then, the ABTS radical solution was diluted with deionized water to reach an absorbance of 0.68–0.72 at 734 nm before being used. The discoloration assay was assessed by mixing 180 µl of ABTS radical solution with 20 µl of various dilutions of each extract in 96-well microplates. The reaction mixture was allowed to stand for 6 min, and the absorbance was measured at 734 nm using a microplate reader. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was use as the positive control due to its antioxidant properties. The scavenging activity of the extracts against ABTS radicals was calculated using the inhibition percentage determined using the formula above and expressed as IC_{50} (µg/ml).

Ferric ion reducing antioxidant power (FRAP) assay

FRAP was evaluated by the measuring Fe (III)/2,4,6-tripyridyls-triazine (TPTZ)-complex using a colorimetric method with a spectrophotometer. The FRAP reagent was prepared using 10 ml of 300 mM acetate buffer and, then adjusted to a pH of 3.6 by adding acetic acid. The acetic acid solution consisted of 1 ml of 10 mM TPTZ in 40 mM HCl, and 1 ml of 20 mM ferric chloride hexahydrate dissolved in distilled water. The ratio of the chloride solution was 10:1:1. The FRAP reagent was prepared fresh daily and was heated to 37 °C for 4 min in a water bath. Crude extracts (10 mg) were placed in centrifuge tubes, diluted to a concentration of 1 mg/ml using absolute ethanol and centrifuged for 1 min. After this process, 20 µl of the sample solution was added to 180 µl of the FRAP reagent, mixed well, and allowed to stand for 8 min at room temperature. The absorbance of the reaction mixture was measured at a 593 nm. The increased absorbance of the reaction mixture indicated a reduction in the activity of the sample. The results were expressed as mg Fe (II)/g and mg Trolox/g of the extracted sample.

Gas Chromatography (GC)-Tandem Mass Spectrometry (MS) (GC-MS/MS) analysis

The chemical compositions of all the extracts were analyzed using a Bruker Scion 436-GC model (Scientific-Instruments, Athens, Greece) equipped with a single quadrupole mass spectrophotometer. Ten microliters of the extracted at a concentration of 3 mg/ml were injected using the Bruker CP-8410 autosampler, at a split/splitless ratio of 1:10. The separation was performed using a 30-m fused silica capillary column with an internal diameter of 0.25 mm and a thickness of 0.25 µm. The carrier gas in the silica column contained a purity of 99.999% helium at a constant flow rate of 1 ml/min. The temperature of the injector in the GC machine was raised to 250 °C, while the ion source in the mass spectrometer was kept at 280 °C. The oven temperature was initially raised from 80 °C to 250 °C at a rate of 5 °C/min and kept isothermal at 250 °C for 4 min. The temperature was then increased to 310 °C at a rate of 20 °C/min and maintained at 310 °C for 15 min, with a total run time of 56 min. The ionization energy for mass detection was 70 eV, with fragments m/z ranging from 40 to 500 Da. The relative amount of each component was calculated as a percentage of the average peak area of this component in the total area. MS Workstation 8 software was used to analyze the data, and the chemical components were identified by comparing the mass spectra with data from the National Institute of Standards and Technology library database.

Statistical analysis

All experimental results were represented in triplicate as mean ± standard error of the mean. A one-way analysis of variance which included Dunnett's test, was used to compare the sample groups and the positive control, which gave a p-value < 0.05. Statistical analyses were performed using Prism Software.

Table 1 The percentage yield of TKP remedy and its plant components.

Scientific name	Common name	Used part	Code	% Yield
A. galanga (L.) Willd.	Greater Galangal	Rhizome	AGE	35.16
B. rotunda (L.) Mansf	Fingerroot	Rhizome	BRE	14.33
O. tenuiflorum L.	Black tulsi	Root	OTE	5.98
–	TKP remedy	–	TRPE	18.85

RESULTS AND DISCUSSION

These studies determined the cytotoxic activity using the SRB assay. Antioxidant activities were determined using the DPPH radical scavenging assay, the ABTS radical cation decolorization assay, and the FRAP. The chemical contents were studied using GC-MS/MS analysis.

Extract preparation

The preparation of medicinal plants extracts for experimental purposes is an initial step and key in achieving quality research outcomes. Alcohol, including ethanol and methanol is universally used in solvent extraction for phytochemical investigation [12]. Previous research reported that a 95% ethanolic extract of BR showed higher cytotoxic activity against cervical cancer cells than in a 50% ethanol or water extract [13]. Moreover, the 95% ethanolic extract of AG showed strong cytotoxic effects on T47D, Henrietta Lacks (HeLa) and WiDr cell lines [14]. Thus, the rhizomes of both the BR and AG and the roots of OT were extracted using 95% ethanol. The result indicated that the 95% ethanolic extract of the TKP remedy had a w/w yield of 18.85%. The percentage yield of all ethanolic extracts of the plant ingredients ranged from 5.98 to 35.16%. AG had the highest percentage yield of the ethanolic extracts, and OT had the lowest. The results are summarized in Table 1.

Antioxidant activity

These studies determined the antioxidant activities of all plants using the DPPH radical scavenging assay. The positive control, BHT showed an IC_{50} value of 9.39 µg/ml. The OT had significantly more vigorous activity than in the positive control, with IC_{50} value of 9.01 µg/ml ($p = 0.017$). The TKP remedy had a lower value than BHT, although it displayed good antioxidant activity. The results are summarized in Table 2.

The ABTS radical scavenging assays of the extracts are shown in Table 2. Both Trolox and BHT positive controls exerted potent activities with an IC_{50} value of 42.60 and 42.11 µg/ml, respectively. The results demonstrated that the OT significantly exceeded the activity of the positive control with an IC_{50} value of 34.43 µg/ml. TKP extract had an IC_{50} value of 72.45 µg/ml.

For the FRAP, the antioxidant activity of the extracts was calculated from a standard curve of $FeSO_4$ presented in units of mg Fe^{2+} per gram of extract (mg Fe^{2+} eq./g). The result indicated that TKP remedy showed antioxidant activity, with a FRAP value of 72.23 mg Fe (II)/g. The highest FRAP value of the plant extracts was OT, and the lowest in AG.

The total phenolic content of all the extracts was determined as milligrams of gallic acid equivalent (GAE) per gram of each extract (mg GAE/g). The OT showed the highest total phenolic content with a value of 38.06 mg GAE/g, followed by the BR with 35.16 mg GAE/g, while the TKP remedy showed 15.24 mg GAE/g.

The 95% ethanolic extract of the TKP remedy and its plant ingredients showed antioxidant activity, with the highest being OT and the second being the TKP remedy. A previous study demonstrated that the methanol extract of OT leaves showed a high activity on both the DPPH and ABTS assays with IC_{50} values of 10.0 and 9.2 µg/ml [15] consistent with this study. This study is the first to report the presence of antioxidants in OT roots. However, TKP had lower antioxidant activity than OT. The combination of OT, BR, and AG improved antioxidant activity. Thus, these compounds have a synergistic effect [16] by enhancing the pharmacological potency of the agents used in TKP remedies.

All extracts showed high activity in the DPPH assay because their IC_{50} was less than that of the ABTS assay. The difference between the radical-scavenging capacities of DPPH and ABTS could be attributed to the solubility and diffusivity of the radicals in the reaction medium. DPPH scavenging is a widely used method for assessing the free radical scavenging activity of natural products [17]. The DPPH assay is conventionally conducted in ethanol, whereas the ABTS assay is performed under aqueous conditions. The ABTS scavenging activity was higher than that of the DPPH assay, which might be because the ABTS radical is more appropriate for determining hydrophilic antioxidants. In contrast, DPPH is more applicable for determining hydrophobic antioxidants [18]. Thus, the plant ingredients of the TKP remedy which contain volatile oil, are appropriate for the DPPH assay. Moreover, an important limitation of the FRAP assay is that it is based on an aqueous solution (acetate buffer); hence, this method is limited to hydrophilic substances. The difference between the two positive controls was that Trolox could be better solubilized in an aqueous solution than in BHT. BHT is soluble in organic solvents [19].

Cytotoxic activity

The cytotoxic activities of the extracts against LS174T colorectal adenocarcinoma cells, human lung adenocarcinoma A549 cells and the MRC-5 human fetal lung fibroblast demonstrated that the TKP remedy showed moderate cytotoxicity in LS174T and A549

Table 2 Antioxidant activity, FRAP value, and total phenolic content of TKP remedy and its plant components ($n = 3$).

Sample	Antioxidant activity (EC_{50} µg/ml)		FRAP value	Total phenolic
	DPPH	ABTS	(mg Fe (II)/g)	content (mg GAE/g)
AGE	59.57 ± 1.09*	>100*	32.25 ± 1.65*	3.65 ± 0.04
BRE	50.25 ± 11.62*	93.93 ± 3.00*	55.80 ± 0.80*	35.16 ± 0.48
OTE	9.01 ± 2.66*	34.43 ± 1.35*	217.80 ± 4.97*	38.06 ± 0.52
TRPE	27.21 ± 4.67*	72.45 ± 1.78*	72.23 ± 0.91*	15.24 ± 0.65
Trolox	NT	42.60 ± 0.94	698.58 ± 3.84	NT
BHT	9.39 ± 1.05	42.11 ± 0.54	298.77 ± 3.44	NT

NT = not test; * significant differences ($p < 0.05$) compared with positive control.

Table 3 Cytotoxic activity of the extracts on the colon adenocarcinoma LS174T cancer cells, the human lung adenocarcinoma A549 and the MRC-5 normal cells ($n = 3$).

Sample	IC_{50}: µg/ml (mean ± SEM)			Selective index	
	LS174T	A549	MRC-5	LS174T	A549
AGE	79.30 ± 1.21	78.85 ± 4.51	71.23 ± 0.33	0.90	0.90
BRE	>100	70.76 ± 4.22	97.37 ± 0.57	NT	1.38
OTE	31.51 ± 0.51	29.60 ± 0.39	37.54 ± 0.51	1.19	1.27
TRPE	52.04 ± 1.42	46.85 ± 1.16	58.00 ± 3.54	1.11	1.24

cell lines, with IC_{50} values of 52.04 and 46.85 µg/ml, respectively. While the OT showed high cytotoxicity activities on the LS174T and A549 cell lines, with IC_{50} values of 31.51 and 29.60 µg/ml, respectively, the other extracts had reduced activity effects. The results are summarized in Table 3.

TKP is an herbal remedy used in Thai traditional pharmacy scriptures to cure the impairment of elements in the body, nourish blood and sexual desires, and is a carminative drug. The three plant components of the TKP have been reported to exhibit cytotoxic activity against cancer cells. One of the three ingredients is AG which can fight breast cancer, hepatocarcinoma, leukemia, and gastric cancer [20]. Kirana et al [21] reported that BR rhizome extract was cytotoxic to HT-29 human colon cancer cell, and MCF-7 human breast cancer cells, and it was cytotoxic to CAOV-3 human ovarian cancer, MDA-MB-231 human breast cancer, and HeLa cervical cancer cells [22]. The OT leaf extract can fight against the leukemic cell line K562 [23], Lewis lung carcinoma [24], and mouth epidermal carcinoma (KB) cells [25].

According to the criteria described by the National Cancer Institute (USA), crude extracts possess high cytotoxic activities with IC_{50} values below 30 µg/ml and moderate activities with IC_{50} values below 50 µg/ml [26]. This study showed that the OT root extract displayed high cytotoxic activity in both LS174T and A549 cell lines. This is the first report on the cytotoxic activity of OT root extract. OT root extract showed high cytotoxicity, which damaged MRC-5 normal lung cells. However, cytotoxicity decreased when OT was used with other herbs in the TKP remedy. The TKP remedy showed moderate cytotoxicity activities in LS174T and A549 cell lines and minor damage to MRC-5 cells. Our results support the traditional Thai theory of using combinations of herbs to treat various diseases.

GC-MS/MS analysis

The chemical compounds present in the TKP remedy were analyzed using GC-MS/MS. Approximately 47 different compounds were observed in the 95% ethanol extracted from the TKP remedy. (S)-4-(1-Hydroxyallyl) phenyl acetate, with a molecular weight of 192 and a chemical formula of $C_{11}H_{12}O_3$, had the most remarkable peak area percent of 16.13 among the 47 compounds detected. The second most significant peak was observed for (E)-4-(3-Hydroxyprop-1-en-1-yl) phenyl acetate, with a molecular weight of 192, a chemical formula of $C_{11}H_{12}O_3$, and a summative peak area percentage of 14.58. The compounds 5-hydroxy-7-methoxyflavanone, 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, and p-coumaroyl alcohol, diacetate had moderate peak area percentages. Their peak areas were 10.73, 8.98, and 5.39%, respectively. $C_{16}H_{14}O_4$/270, $C_{10}H_{18}O$/154, and $C_{13}H_{14}O_4$/234 are the chemical formulas and molecular weights, respectively. A chromatogram of the 95% ethanolic extract of TKP remedy is shown Fig. 1. The major compounds with the top ten highest peak area percentages are listed in Table 4, and other minor compounds with lower peak area percentages are shown in Table S1.

(S)-4-(1-Hydroxyallyl) phenyl acetate and (E)-4-(3-Hydroxyprop-1-en-1-yl) phenyl acetate both of which are natural products found in *A. galanga* had the most remarkable peak area percentage among the 47 compounds detected. The responsive markers of the TKP remedy may be due to these chemicals because AG gives the highest percentage of yield extract. A previous study identified that (S)-4-(1-Hydroxyallyl) phenyl

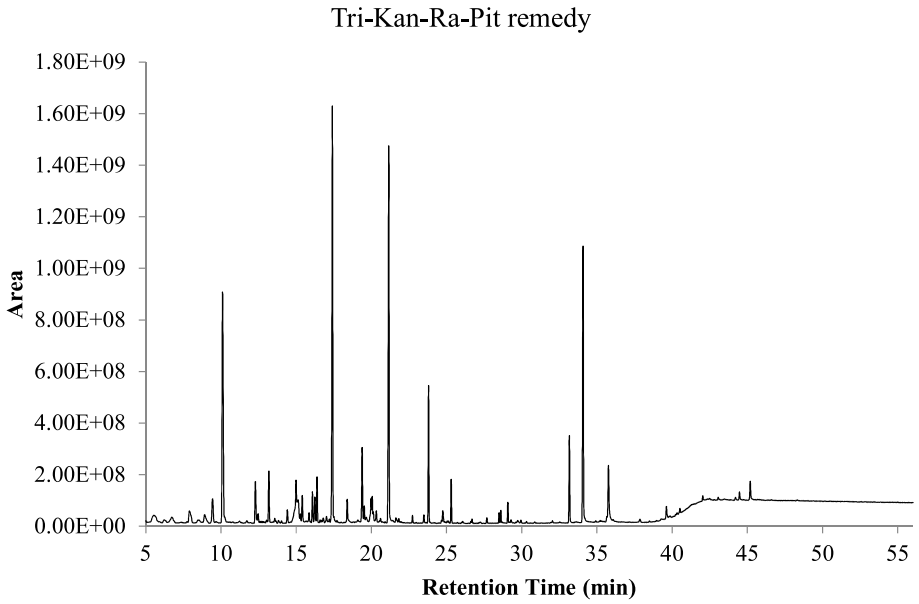

Fig. 1 GC-MS Chromatogram of the 95% ethanolic extract of TKP remedy.

Table 4 Major phytocomponents (top ten) identified in the ethanolic extract of TKP remedy by GC-MS.

No	R/T	Compound	Molecular formula	MW (g/mol)	Peak area (%)
1	10.10	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	154	8.98
2	13.18	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$	196	2.12
3	16.38	.beta.-Bisabolene	$C_{15}H_{24}$	204	1.89
4	17.41	(S)-4-(1-Hydroxyallyl)phenyl acetate	$C_{11}H_{12}O_3$	192	16.13
5	19.38	Phenol, 4-(3-hydroxy-1-propenyl)-	$C_9H_{10}O_2$	150	3.02
6	21.15	(E)-4-(3-Hydroxyprop-1-en-1-yl)phenyl acetate	$C_{11}H_{12}O_3$	192	14.58
7	23.81	p-Coumaroyl alcohol, diacetate	$C_{13}H_{14}O_4$	234	5.39
8	33.17	(E)-4-Methoxy-6-styryl-2H-pyran-2-one	$C_{14}H_{12}O_3$	228	3.47
9	34.08	5-hydroxy-7-methoxyflavanone	$C_{16}H_{14}O_4$	270	10.73
10	35.77	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	2.33

acetate exhibited anti-melanogenic activity and cytotoxicity against theophylline-stimulated mouse B16-4A5 cells [27]. 5-hydroxy-7-methoxyflavanone and 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, are mostly found in *B. rotunda* [28] and *O. tenuiflorum* [29], which have second and third percentages yield extracts, respectively. However, responsive markers will be studied in the future with suitable separation mechanisms in chromatography such as high-performance liquid chromatography.

CONCLUSION

Natural products are rich sources of valuable medicinal agents. This study demonstrated that the TKP remedy has significant cytotoxicity and antioxidant activity. Moreover, this study is the first to report the presence of antioxidants in the *O. tenuiflorum* root. In addition, the data described in this study revealed the cytotoxic and antioxidant properties, which correspond to traditional Thai theory of using herbal recipes to treat various diseases. However, further investigation into its mechanism of action and pharmacological activities is needed for future healthcare benefits.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2023.096.

Acknowledgements: This work was supported by a Naresuan University research fund. The authors are grateful to the Faculty of Public Health, Naresuan University and the Faculty of Medicine, Thammasat University for providing instruments.

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Appendix A. Supplementary data

Table S1 Minor phytocomponents identified in the ethanolic extract of TKP remedy by GC-MS.

No	R/T	Compound	Molecular formula	MW (g/mol)	Peak area (%)
1	5.55	Eucalyptol	$C_{10}H_{18}O$	154	0.42
2	6.23	Maltol	$C_6H_6O_3$	126	0.24
3	6.73	2-Chlorocyclohexanol	$C_6H_{11}ClO$	134	0.35
4	7.91	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	$C_{10}H_{16}O$	152	0.59
5	8.47	endo-Borneol	$C_{10}H_{18}O$	154	0.24
6	8.90	.alpha.-Terpineol	$C_{10}H_{18}O$	154	0.44
7	9.45	Benzofuran, 2,3-dihydro	C_8H_8O	120	1.05
9	12.29	(1S,4R,5R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl acetate	$C_{12}H_{20}O_3$	212	1.71
10	12.47	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$	134	0.48
12	14.42	Caryophyllene	$C_{15}H_{24}$	204	0.63
13	14.99	(E)-.beta.-Famesene	$C_{15}H_{24}$	204	1.77
14	15.13	Sucrose	$C_{12}H_{22}O_{11}$	342	1.02
15	15.40	(S)-4-(1-Hydroxyallyl)phenyl acetate	$C_{11}H_{12}O_3$	192	1.18
16	15.85	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	1.69
17	16.08	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.	$C_{15}H_{24}$	204	1.32
18	16.24	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7	$C_{15}H_{24}$	204	1.12
20	17.01	Phenol, 2-ethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_2$	178	0.38
22	18.39	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	1.03
24	19.52	(S)-1-(4-Acetoxyphenyl)propyl acetate	$C_{13}H_{16}O_4$	236	0.78
25	19.97	Penta-2,4-dienoic acid, 5-(2-furyl)-3-methyl	$C_{10}H_{10}O_3$	178	1.07
26	20.06	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	1.14
27	20.12	8-Heptadecene	$C_{17}H_{34}$	238	0.55
28	20.33	L-Phenylalanine, N-acetyl	$C_{11}H_{13}NO_3$	207	0.59
30	21.63	Penta-2,4-dienoic acid, 5-(2-furyl)-3-methyl	$C_{10}H_{10}O_3$	178	0.32
31	22.73	Tetracosane	$C_{24}H_{50}$	338	0.42
32	23.49	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (E,E)-	$C_{17}H_{28}O_2$	264	0.43
34	24.74	Lidocaine	$C_{14}H_{22}N_2O$	234	0.59
35	25.30	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.79
36	27.67	Hexadecanoic acid, methyl ester	$C_{14}H_{24}O$	208	0.32
37	28.49	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.53
38	28.61	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.61
39	29.08	Methyl stearate	$C_{19}H_{38}O_2$	298	0.92
43	39.62	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	0.76
44	40.51	Eicosanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{27}H_{50}O_6$	470	0.68
45	42.03	m-Tolualdehyde O-pentafluorophenylmethyl-oxime	$C_{15}H_{10}F_5NO$	315	1.17
46	44.48	Stigmasterol	$C_{29}H_{48}O$	412	1.32
47	45.19	.beta.-Sitosterol	$C_{29}H_{50}O$	414	1.73