Thai Medicinal Plant Recipes: Evaluative Study Onanti-proliferative on Cancer Cell Lines and Anti-oxidant Activities

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**ABSTRACT—*This research deals with the in vitro evaluative study of six Thai medicinal plant recipes (i.e. TCRs 1-6) individually undergoing four extraction methods including aqueous cold maceration (AQC), aqueous hot maceration (AQH), ethanolic cold maceration (EtCM) and ethanolicsoxhlet extraction (EtSE), for the antiproliferative activity on four cancer cell lines (i.e. DU145, MCF-7, HeLa, and B16F10) using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and the antioxidant behavior using various colorimetric methods. The plant extracts of most of the six recipes exhibited IC50>100µg/ml on the cancer cell lines, except for TCR5 whose AQC and EtCM respectively showed the IC50 of 53.89 and 75.85µg/ml on B16F10. Besides, the TCR4-AQC and AQH extracts exhibited the best scavenging performance with an SC50 of 0.02 mg/ml, which was 1.5 times higher than vitamin C with an SC50 of 0.03 mg/ml. In the inhibition of lipid peroxidation activity, LC50 of the TCR3-EtSE extract was 0.43mg/ml, approximately 3.4 times the reference standard Vitamin E with an LC50 of 1.44 mg/ml. The findings also revealed that the aqueous extractions of TCR1 had the best ferric ion chelating activity with MC50 of 0.28 - 0.29 mg/ml, roughly 1.6 times higher than the reference standard EDTA with an MC50 of 0.45 mg/ml. In addition, the phenolic contents were highest in the TCR1-AQC extract (2.98 mg/g GAE) and lowest in the TCR3-AQH extract (0.25mg/g GAE). The results also indicated a mild positive correlation between scavenging activity, lipid peroxidation inhibition and total phenolic content. Due to the significantly greater antioxidant efficacy vis-à-vis the antiproliferative activity, the proposed recipes could suitably be applied to the treatment of oxidant-related ailments.*

Keywords—Anti-proliferative, Anti-oxidation, Free radical scavenging, Lipid peroxidation, Metal chelating

1. INTRODUCTION

The pharmacologically active compounds inherent in plants and plants-derived products, particularly those with chemotherapeutic values, render these medicinal plants an alternative treatment for cancer patients. In Thailand, the medicinal use of plants has been commonly accepted and applied in a wide range of practices [1]. For instance, for more than 700 years the people of Lanna (i.e. the present-day Northern Thailand) have been known for their folkloric medicinal wisdom whereby plants are used for treatment of diseases [2]. In addition, a number of Thai plants have been documented for their pharmacological activity and further developed commercially into drugs [3].According to the report, Thailand is undergoing an epidemiologic transition in which the mortality rate attributable to infectious diseases falls while that attributable to chronic conditions, in particular cancers, rises [4]. This fact calls for urgent intervention for cost-effective and safe treatment of cancer, including the use of plants. Interestingly, medicinal plant recipes of the Thai Lanna region have long been safely utilized in the treatment of cancer patients with tangible success [2].

Cancer patients not only suffer the physical and mental pains but also risk impoverishment as a result of the prohibitive treatment costs and loss of incomes [5]. To address, safer and more cost-effective alternatives for the treatment of cancer relative to the exorbitant modern treatment methods should thus be sought, and the use of medicinal plants is one good candidate. Nevertheless, in Thailand many herbal formulas have been used as complementary medicines with no proven evidence of their scientific effectiveness [6]. This current research has thus attempted to scientifically evaluate six Thai medicinal plant recipes for their anti-proliferative oncancercell lines and antioxidant activities.

2. MATERIALS AND METHODS

2.1 Plant authentication and extraction

Authentication: In this research, the selection of plants for each of the six recipes (**Table 1**) was in accordance with the respective recipes' plant lists stored in a database of the Thai Traditional Medicine College of Rajamangala University of Technology Thanyaburi (RMUTT). The plants were subsequently acquired from local herbal vendors. Parts of the selected plant were cut with a sharp knife or machete,washed with tap water, and air dried at room temperature for 2 days. The plant materials were dried at 50°C in a hot air oven, and ground using a stainless steel grinderinto powder.

Extraction: After the acquisition, the plants/materials were washed and milled prior to extraction. In the extraction, the aqueous cold maceration (AQC), aqueous hot maceration (AQH), ethanolic cold maceration (EtCM) and ethanolicsoxhlet extraction (EtSE) were deployed for extraction at a ratio of 1:10 w/w. For the cold maceration (i.e. AQC, EtCM), the six recipes (**Table 1**) was dissolved in either distilled water (for AQC) or 95% ethanol (for EtCM), and placed on a shaker for 24h. On the other hand, the hot extraction (i.e. AQH) was carried out by dissolving each of the six recipes in distilled water prior to boiling for 2h using a hot plate, while each of the medicinal herbal recipes was extracted in 95% ethanol for 12h using soxhlet apparatusfor EtSE. The extracts were subsequently filtered through Whatman™ no. 1 filter papers connected to a vacuum pump. The filtrates were then dried using a rotary evaporator, and the semi-dry extracts were retained in brown bottles and stored at -20°C until use. In this research, the combination of six medicinal recipes (i.e. TCRs 1-6) and four extraction methods (i.e. AQC, AQH, EtCM, EtSx) gives rise to a total of 24 semi-dry extracts.

2.2 Anti-proliferative activity on cancer cell lines and cytotoxicity on human fibroblasts

In the anti-proliferative experiment, this research used the MCF-7 cell line for breast cancer, DU145 cell line for prostate cancer, HeLa cell line for cervical cancer, and B16F10 melanoma cell line, whereas human fibroblasts were used for cytotoxicity assay. All cancer cell lines and human fibroblasts were obtained from the US American type culture collection (ATCC). The experimental cancer cells were retained in DMEM (Gibco BRL, Gaithersburg, USA) supplemented with 10% foetal bovine serum FBS (Gibco BRL, Gaithersburg, USA), 100 IU/ml of penicillin and 100 mg/ml of streptomycin (Gibco BRL, Gaithersburg, USA) at 37°C in 5% CO_2 atmosphere.

The anti-proliferative activity on the four cancer cell lines and cytotoxicity on human fibroblasts was determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as previous described [7]. Specifically, 1×104 cells/well each of the experimental cancer cell lines were seeded into a 96-well plate prior to adjusting to 180µl with DMEM and incubation at 37°C under 5% CO_2 atmosphere for 24h. The cancer cells were then treated with 20µl of the semi-dry extracts individually at 1mg/ml for the initial screening and at various concentrations for the determination of IC_{50}. The treated cancer cells were then incubated at 37°C in 5% CO2 atmosphere for 48h. After incubation, the medium was removed and the cells were washed three times with PBS. Then, 100µl of 0.5mg/ml MTT solution in PBS (pH 7.6) was added into each well and incubated for another 3h. afterward, the MTT solution was removed and 100µl of dimethyl sulfoxide was added into each well to dissolve the blue-violet crystals. The plates were manually shaken for 5 min prior to the measurement of optical density at 570nm using a microplate reader. The percentage inhibition was calculated using the following formula: % inhibition = [100-{A570 of sample x100}/A570 of control]. The concentration providing 50% inhibition (IC_{50}) values were obtained by plotting a graph of % inhibition against concentrations.

2.3 Phytochemical Screeningand total phenolic compounds byFolin-Ciocalteu assay

In this research, the qualitative phytochemical screening of the extracts was carried out using the conventional chemical methods described [8-10].Total phenolic contents of the semi-dry plant extracts were determined by Folin-Ciocalteau reagent in accordance with the method [11] with minor modifications as documented [12]. Specifically, 20µl each of the semi-dry extracts was mixed with 100µl of 1:10 Folin-Ciocalteu reagent, followed by the addition of Na_2CO_3 (80 µL, 7.5%). The assay was carried out in a microplate. The measurement of absorbance at 600nm was taken after 2-hour incubation at room temperature in the dark. In this experiment, Gallic acid was used as the standard reference. TPC was expressed as mg Gallic acid equivalents per gram of dried extract (mg GAE g-1) (Gallic acid equivalent).

Table 1: Compositions and route administrations of the Six Thai medicinal plant recipes.

s/no	Recipe	Plants/Materials	Family	Part use	Ratio of recipe	"Conventional" method of preparation	Route of administration
1.	TCR1	*Smilax corbularia*Kunth *Smilax glabra*Roxb *Albiziaprocera* (Roxb.) Benth *Diospyrosmollis* Griff *FicusHispida* Linn.	Smilacaceae Smilacaceae Mimosaceae Ebenaceae Mimosaceae	Bark Bark Wood Stem Stem	Equal amount	Boiling	Oral
2.	TCR2	*Crateva adansonii*DC *Smilax corbularia*Kunth *Smilax glabra*Roxb Potassium chloride Alum Sulphur powder	Capparidaceae Smilacaceae Smilacaceae	Bark Bark Bark	2:2:2:1:2:2	Boiling	Oral
3.	TCR3	*Datura metel* Linn. *Hydnocarpus anthelminthicus* Pieere ex Laness Curcuma zedoaria (Berg.) Roscoe	SolanaceaeFlacourtiaceae Zingiberaceae	Whole plant Seed Rhizomes	Equal amount	Infusion	Topical
4.	TCR4	*Smilax corbularia*Kunth *Smilax glabra*Roxb Sulphur powder *Suregadamultiflorum*A.Juss *Stemonatuberosa* Lour. *Ananascomosus* (Linn)	Smilacaceae Smilacaceae Euphorbiaceae Stemonaceae Bromeliaceae	Bark Bark Wood Root Leaf	2:2:2:2:2:1	Boiling	Oral
5.	TCR5	*Smilax corbularia*Kunth *Smilax glabra*Roxb Acanthus ebracteatusVahl *Rhinacanthusnasutus* (Linn.) *Nelumbonuclfera*Gaertn *Hedyotiscorymbosa* (Linn.) Bombaxceiba Linn. *Orthosiphonaristatus* (Blume)	Smilacaceae Smilacaceae AcanthaceaeAcanthaceae NelumbonaceaeRubiaceae BombacaceaeLabiatae	Bark Bark Twig Twig Stem Whole plant Stem bark Whole plant	2:2:2:2:2:2:1:1	Boiling	Oral
6.	TCR6	*Stemonatuberosa* Lour *Smilax corbularia*Kunth *Smilax glabra*Roxb Sodium chloride	Stemonaceae Smilacaceae Smilacaceae	Root Bark Bark	2:2:2:1	Boiling	Oral

Note:TCR denotes Thai Anti-Cancer Recipe

2.4 Anti-oxidative activities

2.4.1 Free radical scavenging activity

The free radical scavenging activity of the extracts was determined using a modified DPPH method described [13]. In the experiment, 100µl each of the semi-dry extracts and 100µl of DPPH solution (0.1mg/ml in ethanol) were added into the wells of a 96-well microplate. The plate was then incubated in the dark at room temperature for 30 min prior to the measurement of absorbance using a microplate reader at 560nm. The experiments were performed in quadruplicate and vitamin C was utilized as the positive standard. The percentage of the DPPH free radical scavenging activity was calculated by the following equation, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample:

$$\% \text{ of DPPH free radical scavenging activity} = [(A_0-A_1)/A_0] \times 100$$

The concentrations with 50% scavenging (SC_{50}) efficacy were determined by plotting a graph of the free radical scavenging percentages against the sample concentrations.

2.4.2 Inhibition of lipid peroxidation activity

The lipid peroxidation inhibition activity of the extracts was assayed by the modified Ferric-thiocyanate method [2]. In the experiment, 50 µl each of the semi-dry extracts was added to 50µl linoleic acid in 50% DMSO followed by 50µl each of (5mM) NH4SCN and (2mM) FeCl2 prior to retaining at room temperature for 1h. The measurement of absorbance was taken at 490nm by the microplate reader. The experiments were carried out in quadruplicate and vitamin E was the positive standard. The percentage of lipid peroxidation inhibition of linoleic acid was calculated as below, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample:

$$\% \text{ of lipid peroxidation inhibition} = [(A_0-A_1)/A_0] \times 100$$

The concentrations with 50% inhibition of lipid peroxidation (LC_{50}) were determined by plotting a graph of the percentages of lipid peroxidation inhibition against the sample concentrations.

2.4.3 Metal ion chelating activity

The metal ion chelating activity of the semi-dry extracts was assayed by the modified ferrous ion chelating method described [2]. Specifically, 100µl each of the semi-dry extracts or standard EDTA, 50µl of 2mM $FeCl_2$ in distilled water and 50µl of 5mM ferrozine were added to the wells of a 96-well microplate. The mixtures were incubated at room temperature for 15 min prior to the measurement of absorbance at 570nm by the microplate reader. All the experiments were carried out in quadruplicate. The percentage of ferrozine–Fe2+ complex formation inhibition was calculated by the following equation, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample:

$$\% \text{ of } \textit{Metal ion chelating activity} = [(A_0-A_1)/A_0] \times 100$$

The concentrations with 50% metal chelating effect (MC_{50}) were determined by plotting a graph of the percentages of metal chelating against the sample concentrations.

2.5 Statistical analysis

The results were presented as the mean ± SD of three independent experiments (n = 3). ANOVA was used for the analysis of the test results (LSD test) at the significance level of *p*-value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Anti-proliferative activity and cytotoxicity of the extracts

In Thailand, medicinal plants have traditionally been a source of cancer-chemotherapeutic agents for treatment of cancer patients [14]. In addition, many Thai plants have been shown to possess anti-carcinogenic properties on various human cancerous cell lines with certain promising prospect [15].In this research, 24 semi-dry extracts belonging to six anti-cancer Thai medicinal plant recipes individually undergoing four different extraction techniques (i.e. AQC, AQH, EtCM, EtSE) were evaluated for the anti-proliferative activity. The anti-proliferative experiments were carried out on four cancer cell lines (i.e. DU145, MCF-7, HeLa and B16F10). Initially, to shortlist the semi-dry extracts, 1mg/ml concentration of each of the semi-dry extracts were tested on the experimental cancer cell lines to determine the percentages of inhibition of the extracts (Table 3). The extracts with at least 60% viability inhibition were then selected for the subsequent determination of concentrations required to produce a 50% inhibition in cell viability (IC_{50}). Of the 24 semi-dry experimental plant extracts undergoing the initial screening, on the DU145 cancer cell line, only seven extracts (an equivalent of 29% of the total), all belonging to the ethanolic extraction method, satisfied the 60% viability inhibition criterion. On the MCF-7 cell line, 11 semi-dry extracts (45.8%) met the criterion, ten of which were of ethanolic extraction and one of aqueous extraction. In addition, nine extracts (37.5%) of ethanolic extraction were anti-proliferatively effective on the HeLa cell line, while 10 semi-dry extracts (41.7%), eight of which were of ethanolic extraction and two of aqueous extraction, were effective on B16F10. The disparities in the anti-proliferative efficacy could be attributed to the unequal capability of different solvents and techniques in extracting the essential components from the plant sources [16-17].

Table 3 The percentage (%) inhibition of the semi-dry plant extracts on four cancer cell lines at 1mg/ml

Recipe	Extract type	Anti-proliferative activity at 1mg/ml			
		DU145	MCF-7	HeLa	B16F10
TCR1	AQC	46.88±2.77	69.06±3.28	44.15±3.87	68.36±2.71
	AQH	33.08±4.61	40.48±3.65	37.88±3.37	NA
	EtCM	81.39±1.67	95.56±1.23	75.97±4.01	77.74±3.96
	EtSE	64.11±2.02	91.41±1.32	72.70±4.55	40.28±2.43
TCR2	AQC	15.37±3.76	33.69±3.56	11.06±3.31	<10
	AQH	24.46±3.54	56.34±3.41	<10	NA
	EtCM	91.51±2.10	82.08±2.89	63.13±3.16	73.38±3.32
	EtSE	39.14±3.35	63.94±2.98	27.34±2.35	57.97±4.42
TCR3	AQC	<10	10.95±5.21	11.85±5.14	22.91±4.11
	AQH	12.21±4.22	53.52±3.22	NA	NA
	EtCM	94.11±1.96	83.49±2.98	91.69±3.11	79.16±3.20
	EtSE	60.38±2.77	69.03±3.18	<10	30.60±2.20
TCR4	AQC	17.81±3.56	36.35±3.76	41.74±3.14	53.50±3.54
	AQH	14.20±2.98	55.35±3.21	NA	32.85±3.79
	EtCM	47.66±3.87	82.05±3.31	57.07±1.37	78.09±3.69
	EtSE	39.76±2.04	88.60±2.19	66.28±2.83	65.05±4.04
TCR5	AQC	54.78±2.07	15.59±4.32	54.13±2.85	62.63±6.01
	AQH	42.83±3.67	29.34±3.21	<10	NA
	EtCM	43.27±4.09	54.02±3.25	59.00±1.68	63.76±2.41
	EtSE	63.02±3.87	46.12±3.97	16.47±1.98	45.26±5.11
TCR6	AQC	14.26±4.23	12.63±4.28	56.32±3.40	29.12±6.38
	AQH	16.46±4.98	47.80±3.11	23.95±4.89	NA
	EtCM	30.07±2.16	64.55±3.02	72.98±1.22	77.84±3.28
	EtSE	57.03±1.97	88.82±1.67	61.05±1.33	81.15±5.17

Note: The four extraction methods including AQC, AQH, EtCM and EtSE were aqueous cold maceration, aqueous hot maceration, ethanolic cold maceration and ethanolicsoxhlet extraction. The anti-proliferative activity was expressed in terms of the percent inhibition of cells growth relative to the DMSO control upon 48hrs of exposure to the extracts. Values are expressed as mean ± standard error of quadruplicate experiments. NA= no activity detected.

The anti-proliferative evaluation to establish the concentrations required to produce a 50% inhibition in cell viability (IC_{50}) revealed that all the semi-dry extracts tested on the DU145 and HeLa cancer cell lines exhibited IC_{50} in excess of 200µg/ml. Nevertheless, the TCR1-AQC and TCR4-EtSE extracts showed IC50 of 181.57 and 147.66µg/ml on the MCF-7 cancer cell line, while the TCR5-AQC and TCR5-EtCM extracts had IC_{50} of 53.89 and 75.85µg/ml on the B16F10 cancer cell line (**Table 4**).Based on the National Cancer Institute (NCI) criteria, plants with an IC_{50} of ≤ 30µg/ml could possibly serve as a source for chemotherapeutic agent for cancer treatment [18-19]. Although none of the experimental semi-dry extracts is yet to satisfy this criterion, two extracts belonging to TCR5 (i.e. TCR5-AQC and TCR5-EtCM) possess promising prospects and warrant further in-depth investigation. According to the report, the mild anti-proliferative activity of the experimental semi-dry extracts could be attributed to certain phytochemicals in the plants, e.g. alkaloids and saponins, which are secondary metabolites with anti-carcinogenic property [20].In addition, all extracts exhibited no cytotoxicity on human fibroblasts at the 15th passage which demonstrated cell growth higher than 90%, indicating of no toxicity on normal cells (data not showed).

Table 4 Anti-proliferative IC_{50} values in µg/ml of the selected semi-dry extracts on four cancer cell lines.

Recipe	Extract type	IC_{50} µg/ml of the extracts on cancer cell lines			
		DU145	MCF-7	HeLa	B16F10
TCR1	AQC	NT	181.57±8.55*	NT	>200
	EtCM	>200	>200	>200	>200
	EtSE	>200	>200	>200	NT
TCR2	EtCM	>200	>200	>200	>200
	EtSE	NT	>200	NT	NT
TCR3	EtCM	>200	>200	>200	>200
	EtSE	>200	>200	NT	NT
TCR4	EtCM	NT	>200	>200	>200
	EtSE	NT	147.66±5.62*	>200	>200
TCR5	AQC	NT	NT	NT	53.89±9.96*
	EtCM	>200	NT	>200	75.85±12.56*
TCR6	EtCM	NT	>200	>200	>200
	EtSE	>200	>200	>200	>200
Cisplastin		22.74±0.51	10.74±2.55	24.09±0.89	15.88±1.42
Doxorubucin		0.04±0.00	0.40±0.10	0.59±0.03	0.04±0.01
Vincristin		0.05±0.00	0.33±0.07	0.06±0.00	0.05±0.00
5-Fluorouracil		6.62±0.31	15.21±6.11	10.90±1.84	2.81±0.39

Note: The results are presented as means ± standard error of quadruplicate experiments. Values less than 200 with an asterisk (*) are significantly different ($p<0.05$) relative to the entire standard reference drugs. NT denotes not tested.

3.2 Phytochemical Screening and total phenolic compounds of the extracts

The qualitative phytochemical screening of the semi-dry extracts revealed the presence of many secondary metabolites that are of direct or indirect benefit to health. These include flavonoids, alkaloids, glycosides, saponins, steroids, tannins and anthraquionones (**Table 2**). Phytochemicals found in the extracts depend on kinds of medicinal plants, solvent and temperature used in the extraction process. The heat labile phytochemicals might be degraded by high temperature and long extraction time [13].

Figure 1 illustrates the total phenolic contents of the entire 24 experimental semi-dry plant extracts. The TCR1-AQC extract exhibited the highest phenolic content of 2.98mg/g GAE, while the TCR3-AQH extract provided the lowest phenolic content of 0.25mg/g GAE.Another significant component of plants that helps in combating diseases is polyphenols. Many biological beneficial effects of polyphenols have been documented, e.g. their anti-oxidative property, a lowering of oxidative stress associated with diseases such as cancer and diabetes [21-22], preventing neuron degenerative diseases such as Parkinson's and Alzheimer's diseases [23].

Table 2 Phytochemical screening results of the 24 semi-dry extracts belonging to the six Thai medicinal plant recipes.

Recipe	Extract type	Alkaloids	Flavonoids	Saponins	Glycosides	Tannins	Steroids	Anthraquinones
TCR1	AQC	-	+	+	+	++	+	-
	AQH	-	+	+	+	+	+	-
	EtCM	-	+	+	+	++	+	-
	EtSE	-	+	+	+	+	+	-
TCR2	AQC	-	+	+	+	+	+	+
	AQH	-	+	+	-	+	+	-
	EtCM	-	+	+	+	+	+	-
	EtSE	-	+	+	+	+	+	-
TCR3	AQC	+	+	+	+	+	+	-
	AQH	+	+	-	-	+	+	-
	EtCM	+	+	+	-	+	+	-
	EtSE	+	+	+	+	+	+	-
TCR4	AQC	+	+	+	+	+	+	-
	AQH	-	+	+	+	+	+	-
	EtCM	+	+	+	+	+	+	+
	EtSE	-	+	+	+	+	+	-
TCR5	AQC	+	++	++	+	+	+	-
	AQH	+	+	+	+	+	+	-
	EtCM	+	+	+	+	+	+	+
	EtSE	+	+	+	+	+	+	-
TCR6	AQC	+	+	+	+	+	+	-
	AQH	-	+	+	+	+	+	-
	EtCM	+	+	+	+	+	+	-
	EtSE	+	+	+	+	+	+	-

Note: *+= present, -= absent.*The four extraction methods including AQC, AQH, EtCM and EtSE were aqueous cold maceration, aqueous hot maceration, ethanolic cold maceration and ethanolicsoxhlet extraction.

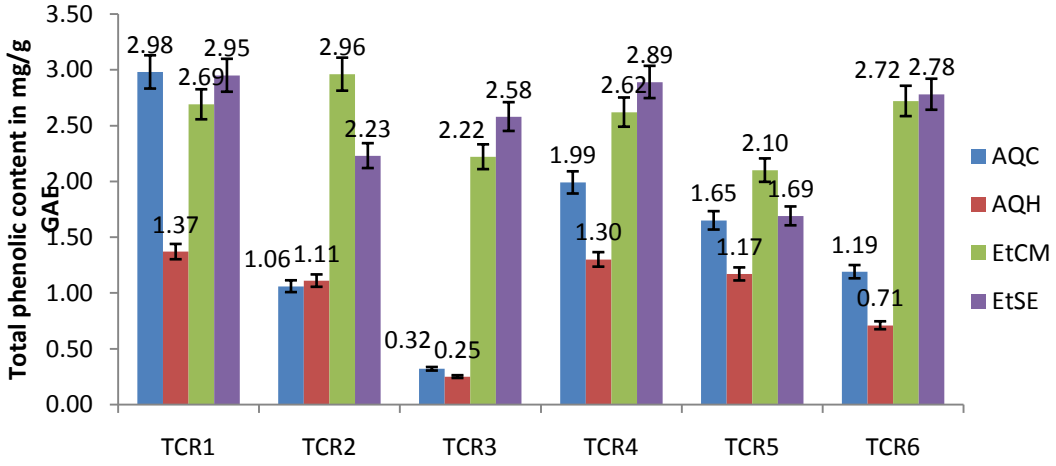

Figure1 Total phenolic contents of the semi-dry extracts belonging to the six Thai medicinal plant recipes (mg/g GAE). The four extraction methods including AQC, AQH, EtCM and EtSE were aqueous cold maceration, aqueous hot maceration, ethanolic cold maceration and ethanolicsoxhlet extraction.

3.3 Anti-oxidative activities of the extracts

It is commonly acknowledged that antioxidants are vital compounds in both health and disease conditions, and inherent in most plants are the antioxidative property. The plant-based antioxidants are of great significance and benefit in the event that the body's internal antioxidant system is so overwhelmed that the failure to prevent oxidant-related degenerative diseases ensues. Many modern illnesses, e.g. cancers, cardiovascular diseases, atherosclerosis macular degeneration, could be attributed to the generation of oxidant compounds in the body, i.e. free radicals [21]. **Table 5** presents the anti-oxidative activities including the radical scavenging activity, inhibition of lipid peroxidation and inhibition of metal ion chelating of the entire semi-dry plant extracts belonging to the six Thai medicinal plant recipes (i.e. TCR1 – TCR6).

Table 5 Antioxidant activities (SC_{50}, LC_{50}, and MC_{50}) in mg/ml of the entire experimental semi-dry extracts

Plants/Recipes	Extract type	Antioxidant activities		
		SC_{50}	LC_{50}	MC_{50}
TCR1	AQC	0.03±0.00	3.32±0.45**	0.28±0.10*
	AQH	0.03±0.01	5.98±0.91**	0.29±0.10*
	EtCM	0.08±0.01**	2.66±0.66**	2.29±0.84**
	EtSE	0.10±0.02**	4.32±0.97**	2.41±0.72**
TCR2	AQC	0.03±0.00	3.32±0.54**	NA
	AQH	0.07±0.00**	6.61±0.96**	NA
	EtCM	0.08±0.00**	9.11±0.99**	6.30±1.28**
	EtSE	0.11±0.00	5.02±0.10**	7.91±0.45
TCR3	AQC	0.05±0.04	0.67±0.17*	NA
	AQH	0.03±0.00	0.72±0.18*	NA
	EtCM	0.03±0.01	0.47±0.16*	14.40±4.30**
	EtSE	0.05±0.01	0.43±0.17*	17.87±1.09**
TCR4	AQC	0.02±0.00	7.49±0.70**	NA
	AQH	0.02±0.01	8.90±0.91**	NA
	EtCM	0.11±0.01**	4.45±0.07**	19.43±0.79**
	EtSE	0.38±0.02**	4.06±0.23**	19.12±2.86**
TCR5	AQC	0.03±0.00	1.28±0.71	NA
	AQH	0.03±0.01	9.53±1.14**	NA
	EtCM	3.38±0.14**	5.24±0.95**	1.00±0.07**
	EtSE	1.44±0.25**	4.71±0.34**	1.54±0.41**
TCR6	AQC	0.07±0.00**	5.67±1.71**	1.45±0.57**
	AQH	0.08±0.00**	8.11±1.80**	0.98±0.33**
	EtCM	0.08±0.01**	8.74±1.41**	NA
	EtSE	0.31±0.01**	8.36±1.34**	NA
Vitamin C		0.03±0.01	NT	NT
Vitamin E		NT	1.44±0.30	NT
EDTA		NT	NT	0.45±0.02

Notes: Values are means ± standard deviations of quadruplicate measurements. Values with an asterisk (*) are significantly lower different, while values with (**) are higher different from the reference standard at $p< 0.05$.NA and NTdenote no activity detected and not tested, respectively.

As shown in the table, all extracts exhibited the free radical scavenging activity and inhibition of lipid peroxidation, whereas only TCR2-5 (AQC and AQH) and TCR6 (ETCM and EtSE) showed no metal ion chelation activity. In addition, the 50% of total extracts outperformed free radical scavenging activity comparableto vitamin C whose SC_{50} is 0.03±0.01mg/ml ($p< 0.05$). In the inhibition of lipid peroxidation, all extracts of TCR3and TCR1 (AQC and AQH) provided the superior result about 3times of the reference standard vitamin E with an LC_{50} of 1.44±0.03mg/ml, and about 2 times of the reference standard EDTA with an MC_{50} of 0.45±0.02mg/ml, respectively.

The reactive oxygen species (ROS) in tissues and cells can cause damage to DNA, proteins, carbohydrates and lipids, thereby rendering it conducive to the development of diseases. According to the report, the deleterious reactions are restrained in part through antioxidants that eliminate ROS and scavenge free radicals [24]. The antioxidative property inherent in some of the experimental semi-dry plant extracts could thus suppress or retard the rapid progression of diseases, e.g. cancers, associated with oxidative stress due to the generation of free radicals.

4. CONCLUSION

For centuries, humans have ingeniously made use of plant materials to cure a plethora of maladies ranging from mild to malignant. Recent growing interest in medicinal plants with a long history of use for treating cancer patients has contributed to extensive research studies and a multitude of extraction techniques to harvest active plant ingredients with antitumor property. In this current research, out of 24 semi-dry experimental plant extracts belonging to six Thai medicinal plant recipes (i.e. TCRs 1 – 6), those from TCR1, TCR4 and TCR5 exhibited the mild levels of anti-proliferative activity on the MCF-7 and B16F10 cancer cell lines. The findings indicated that these extracts could be

potentially developed further into alternative anti-cancer agents despite their performances that are yet to meet the NCI's criteria. More importantly, most of the experimental semi-dry extracts possessed a remarkable anti-oxidative efficacy, especially with regard to the radical scavenging and inhibition of lipid peroxidation, which could thus serve as adjuvant in the post-chemotherapy and help in preventing degenerative diseases, such as Parkinson's and Alzheimer's diseases.

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