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THE ANTIOXIDANT ACTIVITY AND CYTOTOXIC ACTIVITIES AGAINST CANCER CELL LINES OF PROMPAK REMEDY

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ABSTRACT

Prompak is an ancient remedy, composed of seven herbal medicines, which is wildly used in Thai folk medicine as an anticancer drug. In this study, we investigate its cytotoxic and antioxidant activities. The Prompak remedy was extracted with 95% ethanol (EtOH) and water. Cytotoxicity against three cancer cell lines as gastric cancer cell line (Kato-III), liver cancer cell line (Hep G2), and colon cancer cell line (SW 480), compared to one normal cell line as human keratinocyte cell line (Hacat) were determined by SRB assay and antioxidant activities by the ABTS and DPPH scavenging assays. The water extract had the higher yield, 9.87 %. The EtOH extract showed significant, dose dependent killing of cancer cells including Kato III, Hep G2 and SW 480 with an IC_{50} of 9.04±2.22, 30.42±1.12 and 65.58±2.39 µg/mL, respectively whereas this extract was found to be non toxic to normal cells (IC_{50} >200 µg/mL). The water extract demonstrated higher antioxidant activities on ABTS IC_{50} value was 49.38±3.29 µg/mL, while the ethanolic extract displayed the highest reducing DPPH free radical with IC_{50} value of 12.90±1.89 µg/mL. To our knowledge, this work is the first study of the cytotoxic and antioxidant activities of Prompak remady extracts. These data support its potential use as an anticancer drug and warrant further preclinical investigation.

INTRODUCTION

Since ancient times, medicinal plants have played a vital role in preserving human health. The use of medicinal plants has increased, notably, in rural areas in developing countries[1]. In Thailand, Thai folk medicine uses commonly medicinal plants as remedies. Many remedies have been documented, and they have used it over hundreds of years as medicine for treatment of numerous ailment. Researchers are highly interested in studying plants with the aim of supporting herbal drugs. Some Thai herbal remedies have been tested for their anticancer properties and some are used as anticancer treatment. Nonetheless, scientific report of many of these remedies is still lacking[2]. The Prompak remedy is a remedy from

Thai scripture and is a cocktail of several herbal medicines used to treat patients with gastric symptoms consistent with gastric cancer. Prompak consists of seed and the aril parts of *Myristica fragrans*, wood of *Euphorbia antiquorum*, seed of *Piper nigrum*, the latex of *Aloe vera*, oleo-gum-resin obtained from *Ferula assa-foetida*)asafetida(and camphor. Moreover, the bark of *Moringa oleifera* is used as vehicle[3]. However, the properties of Prompak remedy, particularly its cytotoxic and antioxidant activities have not yet been fully investigated. Thus, the objective of this study was to evaluate its cytotoxic activities, using gastric human cell lines, and its antioxidant activities.

MATERIALS AND METHODS

Collection of plants: The plants were purchased from a licensed traditional medical drug store between January and May 2018 in Nakorn Pathom, Thailand. The plant parts were air-dried at 45°C using a hot air oven. Then, the plants were blended using a laboratory mill.

Preparation of the crude extracts

Ethanolic extract: The remedy was mixed with eight medicinal plants. The powdered medicine)200 g(was extracted with 600 mL 95% ethanol)EtOH(for 72 hours. Then the extract was filtrated through Whatman No. 1 filter paper and the ethanol filtrates removed with a rotatory evaporator and kept at 45°C until they were completely dry.

Water extract: The remedy)200 g(was boiled in water)1000 mL(for 15 min. After filtration through a Whatman No. 1 filter paper, the water extract was dried using a freeze dryer.

Calculating Percent Yield

Yields)%; w/w(of each of the extracts were calculated as the ratio of the weight of the extract to the weight of the recipe powder and presented in Table 1. Next, dried extracts were stored in a sterile screw-capped bottle at -20°C until analyzed.

In vitro assay for cytotoxic activity

Human cell lines: Kato III and SW 480 cells were cultured in RPMI 1640 medium supplemented with 10% heated foetal bovine serum and 50 IU/mL of penicillin and 50 µg/mL of streptomycin. Hep G2 cells were cultured in DMEM medium supplemented with 10% heated foetal bovine serum and 50 IU/mL of penicillin and 50 µg/mL of streptomycin. These cells were maintained at 37°C in 5% CO_2 at 95% humidity.

Sulforhodamine B)SRB(assay: The SRB assay developed by Dechayont et al., 2017[4]. Kato III, SW 480 and Hep G2 were seeded at a density of 5×10^4 cells and 3×10^4 cells in a 96 well plate overnight before being treated for 72 hours with different concentrations of ethanol and water extracts of Prompak. Then they were washed and incubated for another 72 h. The experiment was carried out at 37°C in 5% CO_2 at 95% humidity. Cytotoxicity was assayed at 7 days post-treatment. The incubation was terminated by the addition of 100 mL per well of cold 40% trichloroacetic acid)TCA, w/v(, which was left for 1 hour at 4°C. The plates were then washed 5 times with water and air dried, followed by the addition of 50 mL of SRB solution 0.4%)w/v(in 1% acetic acid and left for 30 minutes at room temperature. SRB binds the protein components of living cells that have been fixed to tissue culture plates by TCA. SRB stain was added to each well and left for 30 minutes. After staining, the unbound dye was removed by washing 5 times with 1% acetic acid and the plates air-dried. SRB was dissolved in 100 µL per well of 10 mM Tris base and the absorbance)Abs(read at 492 nm. The percentage of inhibition = 100 x)Abs control – Abs sample(/ Abs control. A fitted dose-response curve was derived by linear regression to calculate the concentration of extract that killed 50% of the cells)IC_{50}(.

Antioxidant activity

DPPH radical scavenging assay: DPPH radical scavenging assay appears as a deep violet colour and shows a strong

absorption band at 520 nm, according to Yamazaki et al., 1994[5]. Briefly, sample solution was pipetted in each concentration in a 96-well plate. DPPH solution was added in each sample and mixed. The absorbance)A(was measured at 520 nm. The percentage inhibition was calculated using following formula.

%inhibition =])Acontrol-Asample(/ Acontrol[X 100

The IC_{50} was calculated by linear regression analysis using GraphPad Prism program.

ABTS radical cations assay: The radical scavenging activity of the extracts against ABTS radical cation was measured using the method of Re et al., 1999[6]. ABTS radical cations)ABTS+(were produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Plant extracts)20 µL(were allowed to react with 180 µL of the ABTS solution and the absorbance was taken at 734 nm after 6 min. The percentage inhibition was calculated using following formula. %inhibition =])Acontrol-Asample(/ Acontrol[X 100. The IC_{50} was calculated by linear regression analysis using GraphPad Prism program.

Statistical analysis: Data of cytotoxicity are presented as mean ± standard deviation)SD(from three to eight independent treatments in individual cell culture wells.

RESULTS AND DISCUSSIONS

The percentage yield was almost tow fold higher in the water compared to the ETOH extract)Table 1(. Only the EtOH extract cytotoxic activity against gastric cancer cell lines)Kato-III(, hepatocellular carcinoma)Hep G2(as well as colorectal adenocarcinoma)SW 480(for an IC_{50} of 9.04±2.22, 30.42±1.12 and 65.58±2.39 µg/mL, respectively. However, the positive control which is curcumin was 5 fold higher potential compared to the ethnolic extract,)IC_{50} 6.47±0.72, 6.34±0.26 and 10.23±1.45 µg/mL, respectively(. Meanwhile, the EtOH extract exhibited low cytotoxicity against normal cell line, with IC_{50} of > 200 µg/mL.

In this sense, the selectivity index is interesting in the case of values greater than 3. To our knowledge, this is the first assessment of the cytotoxic and antioxidant properties of Prompak remedy. Our results show that the EtOH possessed cytotoxic activity but no antioxidant activity and the reverse was true for the water extract. However, both activities were less than those for the two positive controls – curcumin)cytotoxic activity(and BHT)antioxidant activity(. The most promising activity, we were able to conclude that Euphorbia antiquorum is majority activity against Kato-III cells[7]. Also, Wang et al., 2012[8] demonstrated a cytotoxic effect against Hep G2 and SW 480 using MTT assay at conc. 200 µg/mL. Each of the extracts showed strong radical scavenging activity against DPPH and $ABTS^+$ radicals. The EtOH extract also possessed the most potent scavenging activity against DPPH radicals (IC_{50} values of 12.90±1.89 µg/mL). However, the water extract had antioxidant activity in ABTS assays, with being the most potent)IC_{50} values of 49.38±3.29 µg/mL(. Nevertheless, it was less potent than the BHT positive control which was ~10 fold higher)IC_{50} 5.12±0.23 µg/mL(. The antioxidant activities were determined by DPPH and ABTS assays. These assays are typically used as screening assays and have a poor correlation with in vivo activity. Not biologically relevant, these assays were thus performed as a preliminary study to estimate the direct free-radical scavenging abilities of the tested extracts. They measure the free radical scavenging abilities of a given compound by measuring its capacity to reduce an oxidant by electron transfer[9]. Only the water extract had antioxidant properties and this may be due to the presence of *Myristica fragrans*. Previous work has showed that the seed and mace of nutmeg have antioxidant activity and can

scavenge free radicals, reduce metal ions and inhibit lipid oxidation[10-12].

Sample	Yield)%w/w(	Cytotoxic activity				Antioxidant activity	
		Kato III IC_{50})µg/mL(	Hep G2 IC_{50})µg/mL(	SW 480 IC_{50})µg/mL(	HaCat IC_{50})µg/mL(	DPPH assay IC_{50})µg/mL(	ABTS assay IC_{50})µg/mL(
Ethanolic extract	9.87	9.04±2.22	30.42±1.12	65.58±2.39	>200	12.90±1.89	73.81±2.03
Water extract	4.51	>100	>100	>100	>200	26.03±0.34	49.38±3.29
Curcumin	-	6.47±0.72	6.34±0.26	10.23±1.45	9.86±0.24	-	-
BHT	-	-	-	-	-	14.80±1.25	5.12±0.23

Table 1 Percentage yields)% w/w(, Cytotoxic and antioxidant activities)n=3(

Each value represents the mean±SD of triplicates; Kato-III = gastric carcinoma; Hep G2 = hepatocellular carcinoma; SW 480 = colorectal adenocarcinoma; HaCat = human keratinocyte cell line; ABTS = 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonique acid; BHT = butylated hydroxytoluene; DPPH = 2,2-diphényl picrylhydrazyl

CONCLUSION

In summary, the EtOH extract of Prompak remedy had moderate cytotoxic activity and the water extract mild antioxidant activity. Also, we report for the first time on the cytotoxic and antioxidant activities of Prompak remedy. Our results support the further development of Prompak remedy as an anticancer drug with e.g. toxicity studies in animal models and studies on its underlying molecular mechanisms of action.

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