Investigation of the protection efficacy of Thai medicinal plants on irradiation-induced plasmid DNA damage

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Abstract. Extracts of Thai medicinal plants have been reported to be natural sources of antioxidants which can inhibit free radicals and have potential for radiation protection. In this study, we investigated the efficacy of five medicinal plant extracts, cinnamon, ginger, white kwao krua, black pepper, and moringa compared to gallic acid, which served as a positive control in the protection of plasmid pET-15b DNA after exposure to gamma irradiation. pET-15b plasmid DNA was irradiated by gamma radiation at 100 Gy with various concentrations of medicinal plant extracts and DNA damage was measured by using gel electrophoresis. The results showed that exposure of pET-15b plasmid DNA to 100 Gy of gamma radiation induced DNA strand breakage and plasmid DNA alteration from a supercoiled to open circular form. Extracts of moringa, cinnamon and ginger significantly reduced plasmid DNA damage by gamma radiation. While black pepper and white kwao krua extracts did not show observable radioprotective effects. The presence of moringa, cinnamon and ginger extracts during irradiation can protect pET-15b plasmid against radiation-induced DNA damage.

1. Introduction

Medicinal plants are widely known as a major source of valuable bioactive compounds that help to promote the quality of human life. Owing to their potential biological activities, they are widely used in the prevention and treatment of diseases. In Thailand, many kinds of medicinal plants are available such as ginger, drumstick and pepper. They can be consumed either in fresh form or developed as extracts and eaten in the form of supplements. These medicinal plants are known as the important sources of antioxidants which inhibit the effects of free radicals that are a major cause of diseases in humans such as cancer and cardiovascular diseases.

Radiation can cause adverse direct and indirect effects on living organisms, particularly, DNA which is an important part of cell causing DNA damage. As the direct effect, it can break the molecular structure of DNA by an energy transfer process. As the indirect effect, it causes the water molecules, which are the major components of the cell, to break down into free radicals that can react with DNA molecules resulting in DNA damage [1]. In terms of medicinal application, ionizing radiation is an important part of cancer treatment to destroy the DNA of cancer cells and kill them. However, radiation can result in adverse side effects on normal cells also in its path.

Several medicinal plants have been reported for their radioprotective activity which has a potential to reduce DNA damage caused by radiation [2]. Their radioprotection efficacy was provided when treated before or after radiation exposure. The use of medicinal plants to evaluate and reduce the harmful...
effects of radiation because they contain a large number of active constituents which has capability for free radicals scavenging and cellular antioxidants increasing that could be leading the mechanisms for radioprotection. Therefore, the radioprotective effects may be correlated to antioxidant activities.

The aim of this study is to investigate the potential of five commonly-used medicinal plants, namely cinnamon, ginger, white kwao krua, black pepper and moringa for radiation-induced DNA damage protection. The method for evaluating DNA damage is based on the disappearance of supercoiled form and the appearance of open circular form of plasmid DNA upon irradiation.

2. Materials and methods

2.1. Materials
The powdered cinnamon (from bark), ginger (from rhizomes), white kwao krua (from tuber), black pepper (from fruit) and moringa (from leaves) were purchased from local market in Thailand. The medicinal plant extracts were prepared by water extraction.

2.2. Preparation of plasmid DNA
pET-15b plasmid DNA (Novagen, USA) was prepared by using competent JM109 E. coli cells (Promega, USA) for transformation. Single colonies were selected from ampicillin-containing agar plates. Plasmid DNA was extracted using a miniprep kit according to the manufacturer’s procedure (GeneJET plasmid Miniprep Kit, ThermoFisher Scientific, USA).

2.3 Preparation of samples for irradiation and exposure to gamma radiation
The various concentrations (0, 1, 2 and 3 mg/mL) of medicinal plant extracts or 0, 5 and 10 mM of gallic acid (as a positive control) were mixed with 1.23 µg/mL pET-15b plasmid DNA. The mixtures were prepared in polypropylene microtubes and exposed to 100 Gy of Co-60 gamma radiation at 2.96 kGy/h (GC-5000, BRIT, India) at Thailand Institute of Nuclear Technology.

2.4 Gel electrophoresis and band detection
The irradiated plasmid mixtures were investigated by gel electrophoresis in using 0.8% agarose in 1xTBE and compared with non-irradiated plasmid DNA. DNA products were stained with ethidium bromide and visualized on a UV transilluminator. The image analysis software for bands detection was ImageQuant™ 350.

3. Results and Discussion
Plasmid DNA consists of three conformations: a covalently-closed circular or supercoiled form, a linear form when both strands of the DNA have been cut or broken at a single location, and an open-circular form where there is a nick in a DNA strand. The different conformations migrate differently on the agarose gel. The supercoiled DNA runs faster than the linear form, while the open-circular form runs slowest. The linear size of pET-15b plasmid DNA is 5,708 bp and its supercoiled form ran at the apparent size of approximately 3,500 bp, and its open-circular form ran above 10,000 bp (Figure 1, lane 1). Exposure of pET-15b to 100 Gy gamma radiation resulted in the disappearance of the supercoiled form and the transition to the open-circular form (Figure 1, lane 2). Radiation exposure has been known to induce phosphodiester bond cleavages which leads to transformation of the supercoiled to the open-circular or the linear form of plasmids. The presence of gallic acid (GA) during irradiation was able to prevent radiation-induced conversion of the supercoiled plasmid, thus preventing DNA damage (Figure 1, lanes 3-4). This observation was similar to the report of [3] and [4] which showed that GA could protect DNA from radiation-induced strand breaks.
Effects of varying concentrations of medicinal plant extracts on gamma-radiation-induced strand breaks of plasmid pET-15b DNA at 100 Gy. Lane 1: control (0 Gy without additives); lane 2: 100 Gy without additives; lane 3-4: 100 Gy with 5-10 mM gallic acid; lane 5-7: 100 Gy with 1-3 mg/mL cinnamon extract; lane 8-10: 100 Gy with 1-3 mg/mL ginger extract; lane 11-13: 100 Gy with 1-3 mg/mL white kwao krua extract; lane 14-16: 100 Gy with 1-3 mg/mL black pepper extract; lane 17-19: 100 Gy with 1-3 mg/mL moringa extract; lane 20: GeneRuler 1 kb DNA Ladder (ThermoFisher Scientific, USA).

When the pET-15b plasmid was exposed to 100 Gy gamma radiation in the presence of 1 – 3 mg/mL of cinnamon, ginger, white kwao krua, black pepper or moringa leaf aqueous extract, varying degrees of DNA damage protection were observed (Figure 1, lanes 5-20). All of the plant extracts being tested resulted in some retention of the supercoiled DNA. However, most of them showed only minimal radiation protection with less than 10% of supercoiled DNA was preserved. Only 3 mg/mL of cinnamon extract and 1 – 3 mg/mL of moringa extract could maintain the supercoiled DNA at greater than 10% of total (Figure 2).

Moringa leaf extract showed the highest protective ability against radiation-induced damage of pET-15b plasmid DNA. The degrees of protection, as measured by the percentages of supercoiled DNA, increased as the extract concentration increased (Figure 2). 3 mg/mL of moringa leaf extract led to the retention of greater than 30% of the supercoiled plasmid. Leaf extract of moringa had been shown to contain high phenolic content such as gallic acid, chlorogenic acid, and ferulic acid [5]. These compounds exhibited antioxidant properties and radicals-scavenging activities which could prevent the effects of free radicals and oxidative damage. Sinha et al. and Rao et al. had shown that moringa leaf extract had efficiently prevented radiation induced oxidative stress and increased survival in mice when exposed to gamma radiation confirming the radioprotective ability both in vitro and in vivo [6-7]. Furthermore, the extract of moringa leaf could reduce the effects of gamma radiation on DNA double-strand breaks in MCF-7 breast cancer cells [8].

Cinnamon is a common spice which contains sinapic acid and cinnamic acid. Both compounds exhibited antioxidant activities that could lead to the protective effect against radiation-induced DNA damage [9, 10]. The radiation protection ability of cinnamon aqueous extract based on the plasmid assay was shown to increase with increasing concentrations of the extract. However, its protective effect appeared milder in comparison to moringa leaf extract with 3 mg/mL led to slightly more than 10% retention of supercoiled DNA. Higher concentrations of cinnamon extract might be needed for greater protective effects.

The presence of up to 3 mg/mL of ginger, white kwao krua and black pepper aqueous extract showed minimal protection of DNA strand breaks with less than 10% retention of supercoiled DNA. The radioprotective activity of the ginger extract was apparent by many reports [11, 12]. For example, mice were given ginger extract before exposure to gamma radiation which resulted in reduced severity of radiation sickness [13]. Although ginger extracts exhibited radioprotective effects in vivo, our result indicated their direct in vitro effects were minimal. Crude extracts of black pepper were also shown to have strong total antioxidant activity [14]. The extracts contained the bioactive compound, piperine that could prevent oxidative stress and has been used for the treatment of diseases [15, 16]. Extracts of white kwao krua have also been shown to exhibit antioxidant activities and could reduce the effects of ionizing irradiation [17, 18]. Although black pepper and white kwao krua extracts showed only some
radioprotective effects in our plasmid assay, the observed protection may be explained by the antioxidant activities present in these extracts.

![Graph](image_url)

**Figure 2.** Effects of varying concentrations of medicinal plant extracts on pET-15b plasmid conformation ratio at 100 Gy. Column 1: control (0 Gy without additives); column 2: 100 Gy without additives; column 3-4: 100 Gy with 5-10 mM gallic acid; column 5-7: 100 Gy with 1-3 mg/mL cinnamon extract; column 8-10: 100 Gy with 1-3 mg/mL ginger extract; column 11-13: 100 Gy with 1-3 mg/mL white kwao krua extract; column 14-16: 100 Gy with 1-3 mg/mL black pepper extract; column 17-19: 100 Gy with 1-3 mg/mL moringa leaf extract.

**4. Conclusions**
This study showed that aqueous extracts of cinnamon, ginger, white kwao krua, black pepper and moringa leaves could protect pET-15b plasmid DNA from gamma radiation-induced strand breaks at varying efficacy. 1 – 3 mg/mL of ginger, white kwao krua and black pepper showed only minimal protection with less than 10% of supercoiled DNA being retained. Moringa leaf extracts at 1 – 3 mg/mL and cinnamon extract at 3 mg/mL showed greater protective effect leading to greater than 10% retention of the supercoiled plasmid. Based on the plasmid assay, moringa leaf extract appeared most effective. Its radioprotective effects and mechanism should be investigated further to support its use in the mitigation of radiation-induced biological damage.

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