# PLANT BASED RADIOPROTECTANT AS AGENT TO EXPAND THE BOUNDARIES OF RADIOPROTECTIVE EFFICACY

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## ABSTRACT

*Exposure to ionizing radiation comes from several natural and man-made sources. Hazardous radiations cause consequential injuries to biological systems. It has been estimated that about two-thirds of biological damage caused by low linear energy transfer (LET) radiation is due to indirect action, therefore, it is a necessity to formulate such pharmacologically dynamic radioprotector that can render protection to human against destructive and damaging outcome of ionizing radiation. Radioprotectant agent is a substance that protect a normal healthy cell against radiation damage, both in intentional and unintentional radiation exposure while at the same time maintaining the sensitivity to radiation damage in tumour cells. Products of plant origin has become an exciting area of research in drug discovery and development based on its therapeutic application and low in toxicity. Most of the herbs and medicinal plants rich with various phytochemicals with antioxidant properties that scavenge free radicals produced due to radiation exposure, thus imparting radioprotective efficacy. This study was aimed to confirm the phytochemical properties, antioxidant activities and free radical scavenging activities of Polygonum minus (P. minus) methanol extract, which contribute to the possible protective effects against gamma irradiation-induced biological damage. The results showed that Polygonum minus, has an antioxidant and free radical scavenging activities, together with its phytochemicals and bioactivities properties thus qualify for further radioprotective studies by in vitro and in vivo methods.*

## ABSTRAK

*Pendedahan kepada sinaran mengion datang dari beberapa sumber semula jadi dan buatan manusia. Sinaran berbahaya menyebabkan kecederaan berbangkit kepada sistem biologi. Dianggarkan bahawa kira-kira dua pertiga daripada kerosakan biologi yang disebabkan oleh sinaran pemindahan tenaga linear rendah (LET) adalah disebabkan oleh tindakan tidak langsung, oleh itu, adalah satu keperluan untuk merumuskan radioprotektor dinamik farmakologi sedemikian yang boleh memberikan perlindungan kepada manusia daripada merosakkan dan merosakkan. hasil sinaran mengion. Ejen radioprotectant ialah bahan yang melindungi sel sihat normal daripada kerosakan sinaran, baik dalam pendedahan sinaran yang disengajakan dan tidak disengajakan sambil pada masa yang sama mengekalkan kepekaan terhadap kerosakan sinaran dalam sel tumor. Produk asal tumbuhan telah menjadi bidang penyelidikan yang menarik dalam penemuan dan pembangunan ubat berdasarkan aplikasi terapeutik dan ketoksikan*

*yang rendah. Kebanyakan herba dan tumbuhan ubatan yang kaya dengan pelbagai fitokimia dengan sifat antioksidan yang menghilangkan radikal bebas yang dihasilkan akibat pendedahan radiasi, sekali gus memberikan keberkesanan radioprotektif. Kajian ini bertujuan untuk mengesahkan sifat fitokimia, aktiviti antioksidan dan aktiviti penghapusan radikal bebas ekstrak metanol Polygonum minus (P. minus), yang menyumbang kepada kemungkinan kesan perlindungan terhadap kerosakan biologi akibat penyinaran gamma. Keputusan menunjukkan bahawa Polygonum minus, mempunyai aktiviti antioksida dan penghapusan radikal bebas, bersama-sama dengan sifat fitokimia dan bioaktivitinya dengan itu layak untuk kajian radioprotektif selanjutnya melalui kaedah in vitro dan in vivo.*

**Keywords:** Ionizing radiation, radioprotective agent, phytochemicals, antioxidant, free radicals, *Polygonum minus*

## INTRODUCTION

Ionizing radiation produce deleterious effects on biological systems. Indirect action on water molecules causing water radiolysis and further generating free radicals leading to DNA, protein, and lipid membrane damage [1]. When an overload of free radicals cannot gradually be destroyed, their accumulation in body produced a phenomenon called oxidative stress. Antioxidant plays an important role in inhibit the oxidation process, preventing and repairing damages caused by oxidative stress [2].

Among chemical radioprotectors (thiols, aminothiols, thiadiazoles, benzothiazoles, etc) that has been tested clinically for radiotherapy, the efficacy is limited by high toxicity and unwanted side effects associated with them [3]. This limitation could probably be overcome using herbal drugs or dietary modifications, which offer an alternative to the synthetic compounds since they are considered either nontoxic or less toxic than their synthetic counterparts. Therefore, the focus has been shifted to the evaluation of the radioprotective potential of plants and herbs [4], and compounds derived from them due to the easy availability and cost effectiveness.

Radioprotective activity of some plants are mostly related to its phytochemical's constituents - and a range of secondary metabolites found in the different parts of the plants [5] also play a role as a phytochemical antioxidant to scavenge the free radical molecules produces from hydrolysis of water molecules during indirect action of radiation [6]. Scavenging of free radicals is the most important mechanism of radiation protection induced by herbal radioprotectors. Free radical scavenging is mediated by several antioxidant enzymes present in cells which ultimately reduce the oxidative stress [6]. A substance with anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, free radical scavenging, or anti-stress properties may act as a potential radioprotector. Pre-clinical studies in the past two decades have shown that some commonly used medicinal plants and their phytochemicals possess radioprotective effects [7].

*Polygonum minus* (Polygonaceae), generally known as 'kesum' in Malaysia is among the most commonly used food additive, flavouring agent and traditionally used to treat stomach and body aches [8]. This study was aimed to confirm the phytochemical properties, antioxidant activities and free radical scavenging activities of *Polygonum minus* (*P. minus*) methanol extract, which contribute to the possible protective effects against gamma irradiation-induced biological damage.

## MATERIALS AND METHODOLOGY

### *Sample collection and extraction*

The fresh leaves *Polygonum minus* (*P. minus*) were collected from Kuala Kangsar, Perak. The soxhlet extraction method were performed on dried and pulverised leaf sample by utilising 6L of methanol at the temperature within $50^0$C - $60^0$C for 20 hours. Later, extracts were dried using rotary evaporator at the pressure of 80 -100 mbar.

Figure 2.1: The fresh leaves of *Polygonum minus* (*P. minus*)

### *Phytochemical screening*

The qualitative phytochemical screening was conducted on 100 mg of methanolic *Polygonum minus* (*P. minus*) extract to identify the presence of alkaloids, saponins, flavonoids, tannins, triterpenes, and steroids. The determination of phytochemical constituents was performed in accordance to Veshalini et al, [9].

### *Total phenolic content (TPC)*

The total phenolic content (TPC) of *P. minus* methanolic extract was determined by utilising the Folin-Ciocalteu reagent [10]. Following the steps outlined for standard gallic acid, the absorbance for each concentration of the extracts was recorded. To determine the amount of phenolics in the extracts, the samples were prepared in triplicate for each analysis, and the calibration curve was plotted using the average value of absorbance. The results were expressed on a fresh weight basis as mg gallic acid equivalents/100 g of sample.

### *Analysis and identification of compound using HPLC*

The Knauer HPLC (Germany) system, that comprises a pump (Maxi-Star K-1000, Knauer, Germany), a degasser, an automated injector, a column oven, and a UV detector, was used to analyse each sample. In a Eurospher-100 C18 column (5 M, 4.6 250 mm), chromatographic conditions were assessed and adjusted for the measurement of the phenolic compounds in samples. At 254 nm, the chromatographic detection was observed.

### *Heavy metal screening*

The heavy metal analysis of methanolic extract of *P. minus* were analysed for the estimation of trace heavy metals including arsenic, cadmium, plumbum and mercury as per described by Mahmood et al., [11].

### *Antioxidant assay*

### *DPPH free radical scavenging assay*

The ability of the antioxidant in methanolic extract of *P. minus* to scavenge free radicals on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was assessed based on the approach suggested by Yang et al. [10]. The percentage of DPPH free radical scavenging was determined using the equation below:

$$\% \text{ DPPH radical scavenging activity} = \{(A_{control} - A_{sample})/A_{control}\} \times 100\%$$

The $A_{control}$ indicates the absorbance of the control, while $A_{sample}$ indicates the absorbance of the extracts/standard.

### *Superoxide free radical scavenging assay*

The stated approach was used by Sharma & Singh [12] to test the superoxide anion scavenging activity. The formula below was used to compute the percentage of superoxide radical scavenging:

$$\% \text{ Superoxide free radical scavenging activity} = \{(A_{control} - A_{sample})/A_{control}\} \times 100\%$$

The $A_{control}$ indicates the absorbance of the control, while $A_{sample}$ indicates the absorbance of the extracts/standard.

### *Oxygen radical absorbance capacity (ORAC) assay*

A fluorescence plate reader (Thermo Fisher Scientific, Waltham, Mass., USA) was used to perform the ORAC test on a 96-well microplate. Each assessment was conducted in triplicates [13].

## RESULTS AND DISCUSSION

Table 4.1: The phytochemical constituents in methanolic extract of *P. minus*.

| Secondary metabolites | Terpenoids | Flavonoids | Saponins | Tannins | Alkaloid | Steroid | Triterpenes |
|---|---|---|---|---|---|---|---|
| Methanolic *P. minus* extract | + | + | - | + | - | + | + |

+ : Present; - : Absent

Table 4.2: The total phenolic compound (TPC) in methanolic *P. minus* extract.

| Sample | TPC (mg GAE/100g) | Positive control (mg GAE/100g) |
|---|---|---|
| Methanolic extract of *P. minus* | 8876 ± 65.8 | Green tea 15351 ± 61.6 |

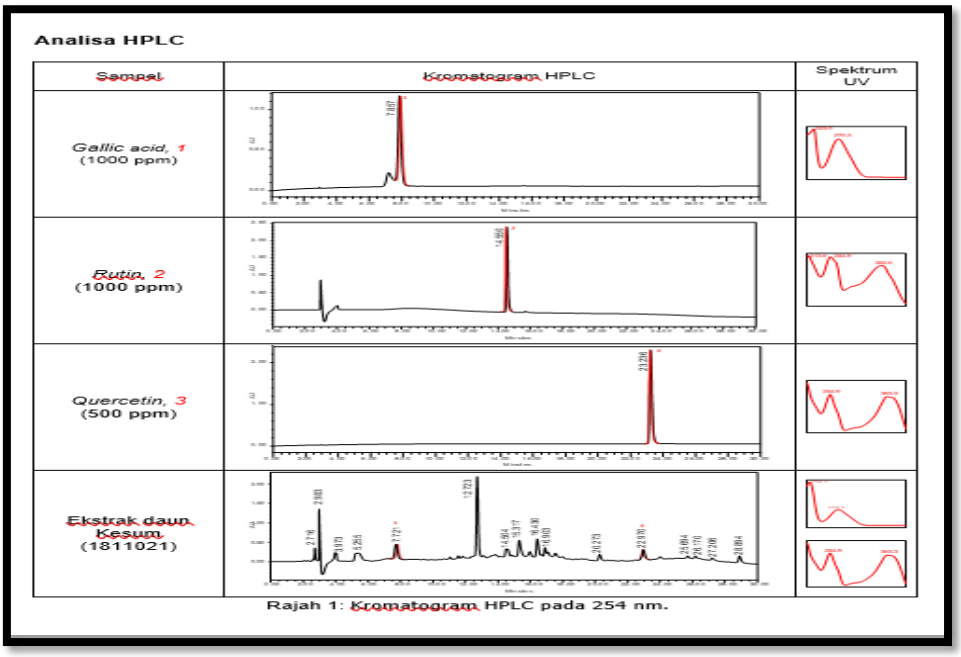


Figure 4.1: The chromatogram of identified phenolic compounds in methanolic extract of *P. minus*.

Table 4.3: Determination of phenolic compound in methanolic extract of *P. minus*.

| Sample | Compound | Average conc. ± RSD (ppm) | Average % in sample ± RSD (w/w) |
|---|---|---|---|
| Methanol extract *P. Minus* | Gallic Acid | 99.67 ± 0.42 | 0.50 ± 0.43 |
| | Quercetin | 20.37 ± 2.12 | 0.10 ± 2.12 |

Table 4.4: Heavy metal trace in methanolic extracts of *P. minus*.

| **No** | **Heavy metal** | **Result (mg/kg)** | **Maximum limit** |
|---|---|---|---|
| 1 | Plumbum (Pb) | 1.57 | < 10.0 mg/kg |
| 2 | Cadmium (Cd) | 0.14 | < 0.3 mg/kg |
| 3 | Mercury (Hg) | 0.06 | < 0.5 mg/kg |
| 4 | Arsenic (As) | 0.05 | < 5 mg/kg |

Table 4.5: The DPPH and superoxide free radical scavenging activity of methanolic *P. minus* extract.

| **No** | **Sample** | **DPPH free radical scavenging activity** | **Superoxide free radical scavenging activity** | **Remark** |
|---|---|---|---|---|
| 1 | Methanolic extract of *P. minus* | 90.09 ± 0.06 (H) | 72.07 ± 1.55 (H) | High (H)- 70- 100%<br>Moderate (M) -50-69%<br>Low (L) – 0-49% |
| 2 | Green tea | 94.01 ± 0.09 (H) | 81.40 ± 0.52 (H) | |
| 3 | Reference | Trolox: 96.61 ± 0.03 | SOD: 72.72 ± 2.07 | |

Table 4.6: The ORAC activity of methanolic *P. minus* extract.

| No | Sample | ORAC Value TE/100g |
|---|---|---|
| 1 | Methanolic extract of *P. minus* | 89,000 |

Herbal goods are preferred to synthetic ones since they are less costly, less hazardous, and safe for humans. The plant's various components include polyphenols, flavonoids, and a wide range of secondary metabolites, that accountable for its radiation protection and anti-cancer qualities [14]. According to previous pharmacological researches, *P. minus* has shown anti-inflammatory, antioxidant, analgesic, antibacterial, in vitro LDL oxidation inhibition, antiulcer, in vitro antiplatelet aggregation, digestive boosting, and cytotoxic activities [8]. Based on the study performed, multiple phytochemical constituents were in the methanolic extract of *P. minus*. These findings is in accordance to literature by Hamid et al. [15], stating flavonoids, phenolic acids, ascorbic acid, aliphatic compounds, organic acids, essential oils, and alkaloids were among the phytoconstituents found in *P. minus*. The flavonoids apigetrin, hyperoside, isoquercetin, astragalin, miquelianin, quercetin, and quercitrin, as well as the phenolic acids gallic acid and coumaric acid, were also available in *P. minus* [15]. Interestingly, the heavy metal analysis showed that the metal trace present in the methanolic extract of *P. minus* is below the range's maximum limit. In addition, *P. Minus* methanolic extract contain high antioxidant and have free radical scavenging activities. ORAC value of the extract was considered higher based on the value of ≥ 10,000 umol TE per day, enough for the maintenance of good health [16]. Previously, it was postulated that *P. minus*'s high polyphenol content, vitamin C, and beta-carotene were to account for its antioxidant action. According to reports, the aqueous, methanol, and ethanol extracts exhibit significant antioxidant capacity that is equivalent to that of synthetic antioxidants such butylhydroxytoluene (BHT) and gallic acid [8].

## CONCLUSION

Methanolic extract of *P. minus* is a promising candidate for radioprotector development with the highlight in antioxidant and radical scavenging mechanism of the radioprotective strategies.

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