

ANTIOXIDATIVE AND ANTIPROLIFERATIVE ACTIVITY OF  
*Polygonum minus* LEAVES EXTRACTS

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*To my beloved family*

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## ABSTRACT

Cancer is one of the leading causes of death in the world, particularly in developing countries. Cancer prevention by dietary constituents has emerged as a novel approach to reduce the number of cancer incidence. The *Polygonum minus* leaves or commonly known as 'Kesum' in Malaysia, have been used as natural remedy in traditional medicine. The leaves of this plant have been reported to be high in antioxidants. Thus, this study was carried out to investigate the presence of phytochemicals, antioxidant effect and antiproliferative activity of *P. minus* leaves extracts. In the present study, ethanol and n-hexane extracts of *P. minus* leaves were examined for the presence of phytochemical constituents using various standard procedures. Folin-Ciocalteu's method was used for the evaluation of total phenolic content of the extracts. The antioxidant activity of the extracts was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals scavenging activity assay. The antiproliferative activity of the extracts was examined against colon cancer cell line (HT 29) using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The ethanolic extract of *P. minus* showed the presence of more phytoconstituents than n-hexane extract. Ethanolic extract showed higher total phenolic content ( $137.07 \pm 29.17$  mg GAE/L) and strong antioxidant effect ( $IC_{50} = 63.1$   $\mu$ g/ml). The n-hexane extract exerted better antiproliferative activity against colon cancer cells ( $IC_{50} = 316$   $\mu$ g/ml). These data shows that ethanolic extract of *P. minus* leaves possesses antioxidant effect and n-hexane extract demonstrated antiproliferative activity against colon cancer cells.

## ABSTRAK

Kanser adalah salah satu punca utama kematian di dunia, terutamanya di negara-negara membangun. Pencegahan kanser menggunakan unsur-unsur pemakanan muncul sebagai satu pendekatan baru untuk mengurangkan jumlah insiden kanser. Daun *Polygonum minus* atau lebih dikenali sebagai 'Kesum' di Malaysia, telah digunakan sebagai ubat semulajadi dalam perubatan tradisional. Kajian telah melaporkan bahawa daun tumbuhan ini mempunyai bahan antioksidan yang tinggi. Oleh itu, kajian ini dijalankan untuk mengkaji kandungan fitokimia, kesan antioksidan dan aktiviti antiproliferatif ekstrak daun *P. minus*. Dalam kajian ini, ekstrak etanol dan n-heksana daun *P. minus* telah dianalisa untuk unsur-unsur fitokimia dengan menggunakan pelbagai prosedur standard. Kaedah Folin-Ciocalteu telah digunakan untuk menilai jumlah kandungan fenolik di dalam ekstrak. Aktiviti antioksidan ekstrak diuji menggunakan ujian kesan radikal bebas DPPH (2,2-Diphenyl-1-picrylhydrazyl). Kesan antiproliferatif ekstrak telah diperiksa terhadap sel-sel kanser usus besar (HT 29) dengan menggunakan ujian MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromida]. Ekstrak etanol daun *P. minus* telah menunjukkan lebih banyak unsur-unsur fitokimia daripada ekstrak n-heksana. Ekstrak etanol menunjukkan jumlah kandungan fenolik yang tinggi ( $137.07 \pm 29.17$  mg GAE/L) dan kesan antioksidan yang kuat ( $IC_{50} = 63.1$   $\mu$ g/ml). Ekstrak n-heksana telah menunjukkan aktiviti antiproliferatif yang lebih baik terhadap sel-sel kanser usus besar ( $IC_{50} = 316$   $\mu$ g/ml). Kajian ini menunjukkan bahawa ekstrak etanol daun *P. minus* mempunyai kesan antioksidan dan ekstrak n-heksana telah menunjukkan kesan antiproliferatif terhadap sel-sel kanser usus besar.

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**LIST OF SYMBOLS AND ABBREVIATIONS**

%	- Percentage
°C	- Degree celsius
<	- Less than
µg	- Microgram
µl	- Microlitre
Abs	- Absorbance
ANOVA	- Analysis of variance
ATP	- Adenosine-5'-triphosphate
CI	- Cytotoxicity Index
CO <sub>2</sub>	- Carbon dioxide
cm	- Centimetre
df	- degree of freedom
DMSO	- Dimethyl sulfoxide

DNA	- Deoxyribonucleic acid
DPPH	- 2,2-Diphenyl-1-picrylhydrazyl
ELISA	- Enzyme-linked immunosorbent assay
<i>e.g.</i>	- <i>exempli gratia</i>
<i>et al.</i>	- <i>Et alia</i>
FBS	- Foetal Bovine Serum
g	- Gram
GAE	- Gallic acid equivalents
h	- hour
HCl	- Hydrochloric acid
HT 29	- Human colorectal adenocarcinoma cell line
IC <sub>50</sub>	- Inhibition concentration at 50%
LN <sub>2</sub>	- Liquid Nitrogen
m	- Metre
M	- Molar
mg	- Milligram
min	- Minute
mL	- Milli Litre
mg/L	- Milligram per Litre
mg/ml	- Milligram per millilitre
mL/g	- Milli Litre per gram
MTT	- Methyl tetrazolium
nm	- Nanometre

OD	- Optical Density
pH	- Hydrogen concentration
PBS	- Phosphate buffer saline
R <sup>2</sup>	- coefficient of determination
RNA	- Ribonucleic acid
rpm	- Revolution per minute
RPMI	- Roswell Park Memorial Institute
SD	- Standard deviation
SPSS	- Statistical Product and Service Solutions
TPC	- Total Phenolic Content
USA	- United States America
UV	- Ultraviolet
v/v	- volume per volume
w/v	- weight per volume

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## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Background of Study**

Cancer is a disease in which abnormal cells divide without control and are able to invade other tissues. Cancer is one of the leading causes of death in the world, particularly in developing countries (WHO, 2013). Lung, liver, stomach, colorectal and breast cancers cause the most cancer deaths every year (WHO, 2012). According to American Institute for Cancer Research (2007), colorectal cancer accounts for over 9% of all cancer incidences. The incidence rates have been rapidly increasing in Eastern Asia for last two decades due to poor dietary and lifestyle factors, including smoking and obesity (Siegel, 2012; Center, 2009). Global awareness of cancer as one of the largest causes of death in people of various ages and racial backgrounds has led to research and many clinical studies in an effort to limit the progression of this disease. Chemoprevention by dietary constituents has emerged as a novel approach to reduce the number of cancer incidence (Soumya, 2011).



*Polygonum minus* or locally named as 'Kesum' or is a traditional plant that has been used by the Malays in the treatment of digestive disorders and stomach pain. *P. minus* originated from Southeast Asia countries namely Malaysia, Thailand, Vietnam and Indonesia. This member of a Polygonaceae family is a raw medicinal plant for preventive health care (Ravichandran *et al.*, 2014). Recently, reports have emphasized that *P. minus* shows high free radical scavenging activity, which is why scientists have become very interested in identifying its phytochemical composition (Suhailah, *et al.*, 2012).

*P. minus* produces a large number of secondary metabolites which includes phenolic compounds such as gallic acid, rutin, coumaric acid and quercetin (Suhailah *et al.*, 2012). The unique flavour of the plant is mainly due to the secondary metabolites (Baharum *et al.*, 2010). The secondary metabolites present in *P. minus* are also responsible for its useful biological properties, which includes antiulcer (Suhailah, *et al.*, 2012), antiviral (Uyub *et al.*, 2010), antimicrobial (Haasim *et al.*, 2013) and antifungal (Johnny *et al.*, 2011) properties. Besides that, it is also being used as shampoo to treat dandruff (Saiful, 2012). Antiproliferative effects of crude extracts of *P. minus* on selected cancer and normal cell lines were studied by Mohd Alfazari and team (2014). Numerous studies had showed good antioxidant activity of *P. minus* (Vimala *et al.*, 2003; Huda-Faujan *et al.*, 2009).

Antioxidant substances are capable of fighting and destroying excess free radicals and repair oxidative damage in biomolecules. The substances act by inhibiting or delaying the oxidation of biomolecules by inhibiting the initiation or propagation of oxidizing chain reactions (Saiful, 2012). Antioxidants are known for their ability in promoting health and lowering the risk for hypertension, heart disease and cancer (Wolfe and Liu, 2003; Valko *et al.*, 2007). In the present study, phytochemical constituents present in the leaves extracts will be screened and antioxidant and antiproliferative properties of the *P. minus* leaves extracts also will be evaluated.

## 1.2 Problem Statement

*P. minus* is being used traditionally to treat rheumatism, indigestion, kidney stones and to control hair dandruff. Researchers have interlinked the pharmacological effects of this plant to its high antioxidant capacity. Aqueous, methanolic and ethanolic extracts of this plant demonstrated high antioxidant activity which was mostly due to its phenolic compounds (Maizura *et al.*, 2011). Fractions from ethanolic and aqueous extract showed gastroprotective effect by inhibiting ulcer lesions in stomach wall of ethanol-induced gastric ulcer in rats (Suhailah *et al.*, 2012) which can protect against the occurrence of colon cancer. Although various chemotherapeutic agents have been developed for the treatment of colorectal cancer, there are harmful side effects caused by the synthetic compounds (Paritala *et al.*, 2014). Thus, dietary interventions have recently caught the attention of researchers and clinicians for the treatment of colon cancer (Center *et al.*, 2009). In this study, the antioxidant property and antiproliferative activity of *P. minus* leaves extract against the colon cancer cells were studied.

## 1.3 Objectives

The objectives of the present study are:

- i. to screen for the phytochemical constituents of *P. minus* leaves extracts,
- ii. to evaluate antioxidant activity of *P. minus* leaves extracts, and
- iii. to investigate antiproliferative property of *P. minus* leaves extracts against colon cancer cells (HT 29).

#### **1.4 Scope of Study**

*P. minus* leaves was extracted using ethanol and n-hexane solvents to obtain polar and nonpolar solvent extracts. The extracts were screened for its phytochemical constituents by various standard procedures to determine the phytochemical constituents or secondary metabolites present in the *P. minus* leaves extracts. The total phenolic content (TPC) of the extracts was determined by using Folin-ciocalteu's method. The extracts were evaluated for its antioxidant effects by employing DPPH assay. The antiproliferative activity of the leaves extracts was evaluated against colon cancer cell line (HT 29) by using MTT assay.

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