

UNIVERSITY TEKNOLOGI MARA

**SAFETY AND EFFICACY OF
*ERYTHROXYLUM CUNEATUM***

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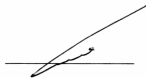
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Abstract

Erythroxylum cuneatum is a member of a tropical plant family which is considered to be a good source of phenolic compounds. The aims of this study were to determine the *in vitro* cytotoxicity, cytoprotective activity, genotoxicity and antioxidant enzymes specific activities of the standardized aqueous extract of *E. cuneatum*. *In vitro* toxicity of *E. cuneatum* extract in four cultured cell lines was determined. The IC₅₀ values in the various cell lines were all higher than 100 µg/ml, indicating low cytotoxic effects. The cytoprotective activity was determined by treating four cultured cell lines (leukemia cells, colon cancer cells, liver cancer cells and normal liver cells) with hydrogen peroxide H₂O₂ (28–158 µM) and determining the effects of *E. cuneatum* (5–50 µg/ml) in the H₂O₂-treated cells. The results showed that H₂O₂ was cytotoxic to cells and the addition of *E. cuneatum* reduced its cytotoxicity. The cytoprotective activity was also determined by treating cultured cell lines (liver cancer cells and normal liver cells) with menadione (2-methyl-1,4-naphthoquinone) (12–30 µM) and determining the effects of the extract (5–50 µg/ml) in the menadione-treated cells. Results showed that the extract, was not able to rescue cells from the cytotoxicity effects of menadione. Genotoxicity of the extract in HepG2 and WRL68 cell lines were assessed by single cell gel electrophoresis (comet assay), whereby cells were treated with hydrogen peroxide (50 µM) as genotoxic control and different concentrations of *E. cuneatum* (0.005–1 mg/ml). A low level of DNA damage was seen at the highest concentration of 1 mg/ml while no DNA damage was seen at the other concentrations. *E. cuneatum* 50 µg/ml when incubated with normal and cancer liver cells significantly increased in catalase specific activity but did not affect cellular GPx and SOD activities. *E. cuneatum* standardized aqueous extract was found to be non cytotoxic and non genotoxic, showed protection to HepG2 and HCT116 cells from H₂O₂-induced toxicity but did alter menadione induced cell death. *E. cuneatum* (50 µg/ml) elevated catalase specific activity in HepG2 and WRL68 cells. More studies are needed if *E. cuneatum* is to be developed as a cytoprotectant.

Keywords: *E. cuneatum*, cytotoxicity, genotoxicity, antioxidant

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CHAPTER ONE

INTRODUCTION

1.1 Overview

As a member of a tropical plant family, *E. cuneatum* is regarded as a good source of phenolic compounds. The plant is from the family *Erythroxylaceae*, a family of four genera, which is particularly found in the tropical areas. The largest genus is *Erythroxylum*, which consists of about 250 species (Brachet *et al.*, 1997). Not much is known about this plant, no research work has been carried out on this plant. What is known is its ethnobotanical use as a stimulant to enhance alertness. The anecdotal evidence was from the indigenous people of Malaysia.

The production of free radicals (FR), which are also present as highly reactive physiological metabolites, has been found to be significantly increased in almost all pathological states, such as inflammation (Dillard *et al.*, 1982), mechanical and thermal injury (Demopoulos *et al.*, 1980; Hall, 1993), aging (Beal *et al.*, 1995), hypoxia–reoxygenation damage (Flamm *et al.*, 1978), malignant growth (Floyd, 1990), and some neurodegenerative diseases (Olanow *et al.*, 1992; Beal *et al.*, 1995; Rego and Oliveira, 2003). These free radicals may be present as reactive oxygen or nitrogen species. An understanding the role of reactive oxygen and nitrogen species mediated pathologies in coronary diseases, cancer, age-related degenerative brain disorders and infectious diseases (Vallejo *et al.*, 2002; Joseph *et al.*, 1999; Aruoma, 1998; Aruoma *et al.*, 2003; Gilani *et al.*, 2000; Ka'hko'nen *et al.*, 2001; Bahorun *et al.*, 2004), have highlighted benefits of the phytochemicals that exert antioxidant actions for human health and prevention of diseases.