UNIVERSITI TEKNOLOGI MARA

ANTIOXIDANT AND TOXICITY STUDIES ON *OCTOMELES SUMATRANA (DATISCACEAE)*

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ABSTRACT

The antioxidant capacity and toxicity of the water soluble extract of the bark of *Octomeles sumatrana (Datiscaceae)*, a medicinal plant known as Binuang by the Kadazandusun communities of Sabah, were investigated. The antioxidant capacity was evaluated by different methods such as photochemiluminesence, measurement of total phenolics, free radical (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP). In addition, the inhibition of liver microsomal lipid peroxidation was assessed by the thiobarbituric acid reactive substance (TBARS) method. Results obtained by the photochemiluminescence method showed the water soluble antioxidant capacity to be 414.2 μg/mg in ascorbic acid equivalents while the lipid soluble antioxidant capacity was 1447.4 μg/mg in trolox equivalents. The total phenolic content was 0.36 ±0.2 mg/mg of extract expressed as gallic acid equivalents. The FRAP assay produced 4.3 ± 0.9 μM Fe^{2+} /mg of extract while the controls, trolox and ascorbic acid, produced 8.6 ± 1.0 μM and 10.5 ± 0.6 μM Fe^{2+}, respectively. The median inhibitory concentration (IC_{50}) for free radical scavenging of DPPH was 4.7 ± 0.6 μg/ml while the IC_{50} values for the controls, trolox and ascorbic acid were 3.9 ± 0.4 μM and 3.8 ± 0.2 μM, respectively. The IC_{50} value for hydroperoxide-initiated lipid peroxidation was 21.6 ± 2.3 μg/ml while for the control, trolox, it was 9.9 ± 1.8 μg/ml. The IC_{50} value for iron(II)/NADPH-initiated lipid peroxidation was 15.4 ± 0.5 μg/ml while for the control, trolox, it was 3.2 ± 0.1 μg/ml. In the xanthine + xanthine oxidase oxidatively stressed mouse model, the extract caused a significant reduction in the heart TBARS and plasma MDA levels as compared to controls Acute, subacute and subchronic toxicity studies were performed on male albino mice while cytotoxicity assays were undertaken using normal (WRL68 liver cell) and cancer (HepG2, MCF7, HCT116 and K562) cell lines. Following acute (a single administration of 0.05, 0.5 or 5 g/kg body weight, p.o.), subacute (daily administration of 0.5 g/kg body weight, p.o. for 7 days) and subchronic (daily administration of 0.05 g/kg body weight, p.o. for 3 months) dosing of *O. sumatrana*, blood was collected for biochemistry and haematological analysis while the heart, liver, kidney and lung were collected for measurement of the product of lipid peroxidation by the TBARS assay. Acute administration of *O. sumatrana* at all doses neither elicited any overt signs of toxicity nor any change in the behaviour of the mice during a 24 hour observation period. Similarly, no signs of toxicity or changes in animal behaviour were seen with subacute and subchronic dosing with the extract. The blood biochemistry, haematology, plasma malondialdehyde (MDA) and organ TBARS levels of the acutely, subacutely and subchronically dosed animals were not different from control values. Cytotoxicity of *O. sumatrana* in human normal and cancer liver cell lines as assessed by the MTS assay showed IC_{50} values in the range of 200-400 μg/ml of extract. In addition, there were no genotoxic effect of the extract on the DNA of cultured cell lines as measured by the comet assay. In summary, the aqueous bark extract of *O. sumatrana* showed high *in-vitro* antioxidant capacity and can be generally regarded as safe, based on no toxicity i.e. no deaths or adverse effects, at 5 g/kg acute dose *in vivo* and on large IC_{50} values (>200 μg/ml) in cytotoxicity tests determined *in vitro*.

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CONTENTS

CHAPTER I

INTRODUCTION

1.1 Overview

Traditional medicine has a long history of serving the people all around the world. WHO estimated 80% of the people around the world especially in the developing countries still rely on traditional medicine for their healthcare (WHO, 2002). Large numbers of the population in developing countries are unable to afford pharmaceutical drugs and they continue to use their own systems of indigenous medicines that are mainly plant based (Phillipson, 2001). Natural products remedies used in traditional folk medicine have been the source of many medically beneficial drugs (Battle et al., 2005). Many of the medicinal plants presenting interesting biological and pharmacological activities have been used as therapeutic agents (Gertsch et al., 2003; Koehn & Carter, 2005; Mann, 2002). Recently, there is an increasing trend, worldwide, to integrate traditional medicine with primary health care. The resurgence of plant remedies result from several factors such as the effectiveness of these remedies, side effects associated with most modern drugs and the development of science and technology which has allowed for extensive research into herbs (Kong et al., 2003). Traditional knowledge and practices have made important contribution to modern medicine as attested by the fact that 122 drugs have been discovered from 94 plant species through examination of their use in traditional medicine (Fabricant & Farnsworth, 2001).

People of the Kadazandusun communities of Borneo Malaysia had rely on traditional medicine handed down to them through the generations even though the practice of modern medicine is wide spread (Ahmad & Holdsworth, 2003). Traditional medicinal plants are used in one form or another to cure or alleviate a