THE ANTIOXIDANT PROPERTIES, SAFETY AND BIOAVAILABILITY STUDIES OF A NEW DEVELOPED PRODUCT - MIXED FRUIT JUICE (MFJ)

BY

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ABSTRACT

The consumption of fruits has been correlated with better health and prevention of several diseases. Phytochemicals such as phenolic compounds and vitamins in fruits are believed to act as antioxidants that could reduce oxidative damage to biomolecules by modulating reactive free radicals. Since Malaysia is rich in a wide variety of tropical fruits, we should examine them in producing healthy and tasty juices. A new fruit juice named Mixed Fruit Juice (MFJ) was formulated from a combination of three tropical fruits (soursop, mango and kasturi lime).There is no information regarding the antioxidant activity, antioxidant compounds, safety parameters and bioavailability of the bioactive compounds in this product. Therefore, this study focuses on the measurement of antioxidant activity by using the common chemical methods (TPC, FRAP, DPPH) followed by the identification and quantification of phenolic acids by GC-MS. The safety and *in vivo* antioxidant effects of MFJ were done in normal and aged rats. Human intervention study was established in order to determine the bioavailability of the phenolic compounds in MFJ. Results show that MFJ contained 23.50±1.57 mg GAE/100g (TPC) and 18.77±0.46 mg TE/100g (FRAP) and DPPH value at 83.66±0.84%. The 28-day sub-chronic toxicological test showed no systemic toxicity attributable to the MFJ administration in normal rats. There was no significant effect in the haematological profiles and both liver and kidney function tests when compared to control rats. Furthermore, MFJ was able to modulate the antioxidant enzymes such as SOD, GPx and CAT in the blood and tissues (liver and brain) of aged rats. Aged condition reduces all the antioxidant enzymes in blood and tissues. The supplementation of MFJ in aged rats had increased the TAS, GPx and SOD values in the blood by 14%, 30% and 104%, respectively. In the liver, the CAT and SOD also increased by 33% and 22%, respectively. In addition, substantial increment was observed in the activity of enzymes in the brains of aged rats. GPx was found to increase by 75% in aged rats after receiving MFJ. Moreover, SOD had increased more than 100% in aged rats after receiving MFJ. In the bioavailability study, phenolic acids (*p*-coumaric acid, ferulic acid and caffeic acid) were found in conjugated form (plasma and urine) and mainly further metabolized to hippuric acid that was excreted in the urine after one hour of MFJ ingestion. Hippuric acid, the main metabolite in urine was found to increase by 25% compared to baseline. In addition, ferulic acid which is known as an antioxidant compound was also recovered in the urine at 16% in one hour. Thus, it is suggested that the absorption of phenolic acids may occur in the small intestine. In conclusion, this current study shows that MFJ was able to exert the antioxidant effects due to the content of phenolic acids which was proven bioavailable in humans.

خلاصة البحث

ارتبط تناول الفواكة مع تحسين الصحة والوقاية من العديد من الأمراض. ويعتقد أن المواد الكيميائية النباتية، مثل: المركبات الفينولية والفيتامينات في الفواكه تكون بمثابة مضادات الأكسدة التي يمكن أن تقلل من الضرر التأكسدي للاالجزيئات الحيوية عن طريق تحوير الجذور الشوارد الحرة. وتُعد ماليزيا دولة غنية بفواكها الاستوائية المتعددةوالذيذة، لهذا كان من المطلوب اكتشاف هذه الثمار بغية إنتاج عصير فواكه صحية ولذيذة. ولهذا تم فحص وتحليل عصير الفاكهة الجديد (MFJ) والممزوج من ثلاثة فواكه استوائية، وهي: (درريان فلندا،والمانجو، وكاستورى الجير). وبالرغم أنه لا توجد معلومات بشأن نشاط مضادات الأكسدة والمركبات، ومعايير الأمن والسلامة والتوافر البيولوجي للمركبات الحيوية النشطة في هذا المنتج،لذا تركزت هذه الدراسة على قياس نشاط مضادات الأكسدة باستخدام الطرق الكيميائية الشائعة (TPC, FRAP, DPPH)، متبوعا بالتحديد، وتقدير الأحماض الفينولية باستعمالGC–MS ،وقد أجري تجاربالسلامة، وتأثيرات مضادات الأكسدة على الجسم الحي في مادة(MFJ) على الفئران العادية والمسنة.وتنشأة دراسة التدخل البشري من أجل تحديد التوافر الحيوي من المركبات الفينولية في مادة(MFJ). وأظهرت النتائج أن(MFJ)قد اشتملت على 23.50 ± 1.57ملغ(TPC) GAE / 100g ،و77.18± 0.46 ملغ / TE (FRAP) 100وقيمةDPPHفي 83.66± 0.84٪. كما أظهر اختبار السمية شبه المزمنة لمدة 28 يوما أنه لا سمية جهازية تعزى إلى الإدارة(MFJ)في الفئران العادية. ولم تكن هناك آثار كبيرة في ملامح الدموية، ووظيفة كل من الكبد والكلى بالمقارنة مع الفئران المراقبة. وفوق ذلك، كان مادة (MFJ) قادرة على تعديل الانزيمات المضادة للأكسدة، مثل: (SOD, GPxوCAT)في الدم والأنسجة (الكبد والدماغ) في الفئران الكبيرة السن. وأن حالة كبر السن يقلل كل الانزيمات المضادة للأكسدة في الدم والأنسجة. وتكملات من مادة(MFJ)في الفئران الكبيرة السن ازداد درجات(TAS، GPx وSOD) في الدم بنسبة 14٪، 30٪ و 104٪ على التوالي. وفي الكبد، ازداد درجة (CATوSOD)أيضا بنسبة 33٪، و 22٪ على التوالي. وبالإضافة إلى ذلك، لوحظ زيادة كبيرة في نشاط الإنزيمات في دماغ الفئران الكبيرة السن. ولوحظ زيادة درجةGPxبنسبة 75٪ في الفئران الكبيرة السن بعد تلقي(MFJ).زيادة على ذلك، قد ازدادت(SOD)أكثر من 100٪ في الفئران الكبيرة السن بعد تلقي(MFJ).وفي دراسة التوافر الحيوي، تم العثور على الأحماض الفينولية (كومازكحامض، حمض الفيرليك وحامض كافيك) على شكل مترافق (البلازما والبول) ومتمثل أيضا على حمض هبيوريك التي تفرز في البول بعد ساعة واحدة من الابتلاع لمادة(MFJ). تم العثور على حمض هبيوريك، المتمثل في البول وازداد بنسبة 25٪ مقارنة مع خط الأساس. بالإضافة إلى ذلك، تم العثور على حمض الفيرليك المعروف باسم مركب مضاد للأكسدة أيضا في البول بنسبة 16٪ بعد ساعة واحدة. وبالتالي، فإنه يقترح أن امتصاص الأحماض الفينولية قد تحدث في الأمعاء الدقيقة. أخيراً، تظهر هذه الدراسةأن مادة(MFJ)قادرة على ممارسة تأثيرات مضادة للأكسدة نظرا لما تحتويه من أحماض فينولية التي ثبت توافرها الحيوي، والبيولوجي في الإنسان.

APPROVAL PAGE

The thesis of Hadijah Hassan has been approved by the following:

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Hadijah Hassan

Signature .....Date .....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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Affirmed by Hadijah Hassan

…… ……..

Signature Date

In dedication to:

My beloved mother, Azizah Binti Yusof

My beloved husband Mahdi Al-Hilmy Bin Arshad

My beloved children

Muhammad Al-Hafiy

Muhammad Al-Haikal

Maisarah Al-Humaira

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LIST OF ABBREVIATIONS

ABTS	2,2-Azino-Di-3-Ethylbenzthiazoiine Sulphonate
AOC	Antioxidant capacity
ANOVA	One-way Analysis of Variance
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BHA	Butylated Hydroxyanisole
BSTFA	N,O-Bis(Trimethylsilyl)-Trifluoroacetamide
CA	Caffeic Acid
CAT	Catalase
CCl_4	Carbon tetrachloride
CHOL	Cholesterol
CREA	Creatinine
COA	Coumaric Acid
COMT	Catechol-*O*-Methyltransferase
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic Acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl Radical-Scavenging Assay
EDTA	Ethylenediaminetetraacetic Acid
ET	Electron Transfer
FA	Ferulic Acid
FRAP	Ferric Reducing Power (FRAP) Analysis
GC-M	Gas Chromatography Mass Spectrophotometry
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GAE	Gallic Acid Equivalent
GR	Glutathione Reductase
H&E	Haematoxylin and Eosin
HAT	Hydrogen Atom Transfer
HGB	Haemoglobin
HTC	Haematocrit
LPO	Lipid Peroxidation
MARDI	Malaysian Agricultural Research and Development Institute
MFJ	Mixed Fruit Juice
MDA	Malondialdehyde
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
ORAC	Oxygen Radical Absorbance Capacity
PLT	Platelet Count
RBC	Red Blood Count
ROS	Reactive Oxygen Species
SD	Sprague-Dawley
SEM	Standard Error of Mean
SOD	Superoxide Dismutase
TAS	Total Antioxidant Status

TSH	Total Sulfhydryl groups
TE	Trolox Equivalent
TG	Triglyceride
TPC	Total Polyphenol Content
TP	Total Protein
TBIL	Total Bilirubin
WHO	World Health Organization
WBC	White Blood Count

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Fruit consumption choices are no longer based only on taste and personal preference, but also a desire for better health. In recent years, there has been an increase in the economic exploitation of the products and by-products of specific fruits that relate to the increasing concern about diet and health status (De Souza et al., 2012). Fruits are source of antioxidant compounds such as phenolic acids, vitamins, carotenoids and minerals that can contribute to the medicinal health effects (Almeida et al., 2011).

Fruit consumption has become a concern of health due to the vital nutrient contents in fruit. Most fruits contain considerable amounts of micronutrients, such as minerals, vitamins, fibers and secondary phytochemicals compounds. Increasing evidence showed the importance of these compounds for the prevention of many human diseases such as cardiovascular, certain type of cancers, inflammation and mascular degeneration and nuerodegenerative problems (Tanaka et al., 2012; Obon et al., 2011; Benetou et al., 2008). Low intake of fruits and vegetables was estimated to cause about 19% of gastrointestinal cancer, 31% of ischaemic heart disease and 11% of stroke (World Health Organization [WHO], 2002).

Among the compounds present in fruit that have received significant attention was antioxidant substances. Antioxidants have been reported to have beneficial health effect against oxidative stress that can prevent a number of chronic diseases (De Souza, 2012; Yahia, 2010). Consuming food rich in antioxidants may help people to increase their antioxidant defense system in the body, thus avoid oxidative damages that could lead to chronic diseases (Beaulieu & Schaefer, 2014). Natural antioxidants

present in fruit have attracted much interest because of their safety, potential nutritional values and therapeutic efficacy.

Research suggested that phytochemicals, specifically phenolic compounds mainly in fruits, may be responsible for the putatively positive antioxidant benefits to human (Crowe & Murray, 2013). Vitamin C is long considered as a major, naturally occurring nutrient and antioxidant in our daily life, however, it may contribute far less to the overall antioxidant capacity of fruit than the phenolic compounds, a prominent class of phytonutrients (Gorenstein et al., 2004). Among the known phenolic compounds is a group of hydroxycinnamates which includes *p*-coumaric acid, ferulic acid, caffeic acid, sinapic acid and their esterified conjugates such as chlorogenic acids that are abundant in fruits, vegetables, coffee and cereals. Studies showed their potential in the prevention of chronic diseases such as diabetes, cardiovascular and cancer (Mancuso & Santangelo, 2014; Jaganathan et al., 2013; Kumaran & Prince, 2010; Jung et al., 2006). However, the impact of these dietary hydroxycinnamates on health depends on their bioavailability *in vivo*. Thus, an accurate measure of dietary intake of phenolic compounds is mandatory (Vetrani et al., 2014).

Based on the growing body of evidences related to the increasing fruit consumption and decreased risks of various chronic diseases, the US Department of Agriculture Dietary Guidelines for Americans 2010 (DGA) recommended increasing the amount of whole fruits eaten daily such that half the plate is composed of these plant foods. A serving fruit is defined as one cup fruit, one cup 100% fruit juice or half cup of dried fruits (Crowe & Murray, 2013).

Eventhough many people know about the benefits of consuming fresh fruits for healthy diet, those who have busy lifestyle would rather take a quick fix in the form of a supplement or pill. Another alternative to eat fresh fruits is by drinking fruit

juice or fruit beverage that can be obtained easily in the supermarket elsewhere. The only thing needs to be considered is the nutritional content of the fruit juices/beverages. As an efficient marketing tool, some manufacturers have labeled their products as a functional drink, healthy drink, high antioxidant juice and many other claims that could attract consumers to buy their products. As a results, these ready-to-drink beverages especially those with the claims of containing high in antioxidants, have become one of the fastest growing food categories on the market (Sloan, 2012).

Emerging evidences suggested that drinking fruit juices may well be effective as consuming the whole fruits in relation to a reduction of the chronic diseases (Mullen et al., 2007). For instance, long-term fruit juices consumption can provide protection against Alzheimer's disease (Dai et al., 2006). Jia et al. (2012) reported that the radical scavenging activity of blackcurrant juice has shown an inhibitory effect on gastric cancer cell proliferation. In addition, an increasing intake of high polyphenol fruit beverages has been shown to decrease serum biomarkers of inflammation and oxidative stress while increasing the serum antioxidants levels (Nemzer et al., 2011). MonaVie Active®, a famous drink which contained a mixture of fruits and berries, including acai berry, was reported to increase the serum antioxidant and decrease lipid peroxidation in humans after 2 hrs drinking of the juice (Jensen et al., 2008). Another study conducted with acai berry was from Udani et al. (2011), which indicate the positive effects in the overweight human by reducing glucose and cholesterol levels. Concord grape juice supplementation also showed an increased verbal memory performance in human subject with mild cognitive impairment (Joseph et al., 2009).

In relation to these positive effects of various fruit juices, MARDI, a Malaysian government research institute in the agricultural field, has focused in a

wide range of tropical fruits that become interesting choices in producing new local fruit juices that contain high antioxidant compounds. This was due to the increasing health consciousness and growing interest in the role of food for maintaining human well-being. Moreover, more people now have less time for cooking and with the increasing of out-of-home food consumption there is a demand for natural products which could contribute to a healthier diet and better quality of life.

Fruit juice is one of the food choices that can contribute to the high intake of antioxidants in our daily diet. Since Malaysia is a tropical country that is planted with a wide variety of delicious tropical fruits, we should explore the health benefits of these fruits. The most common fruits available in our country are mango, banana, papaya, guava, pineapple, durian and others. They are normally consumed in fresh form or turned into processed products such as juice, jam, chips and pickles (Zakaria et al., 2012).

MARDI has formulated a new fruit juice namely Mixed Fruit Juice (MFJ) from a combination of three selective tropical fruits, soursop (*Anona muricata* Linn), mango (*Mangifera indica* var Chokanan) and kasturi lime (*Citrus microcarpa*). The selections of these fruits were based from previous projects related to the screening of various local tropical fruits for high antioxidant sources. The compounds of interest were free phenolic acids, a relatively small molecular weight such as *p*-coumaric acid, ferulic acid and caffeic acid. These compounds were selected because of their known antioxidant activity that shows some positive health promoting effects reported in other different studies.

There were no data available on its antioxidant capacity in vitro and the quantity of antioxidant compounds in MFJ. MFJ also needs to be tested for its antioxidant efficacy and bioavailability profile through in vivo studies. Furthermore,

there are very limited data found in the literature regarding the area of bioavailability study from tropical fruit or tropical fruit juice. The scientific information is very important because MFJ is aimed to be commercialized in the Malaysian market. Based on all the literature reviews mentioned above, this study was conducted to provide scientific information on the safety, antioxidant capacity and the bioavailability studies of MFJ. The present studies provide novel information about MFJ, its safety, efficacy, bioactive compounds and bioavailability profiles. All these are essential in understanding its antioxidant potential and future application for human consumption.

1.2 GENERAL OBJECTIVE

To obtain scientific evidences on the antioxidant activities, safety parameters and bioavailability of a new developed fruit juice - Mixed Fruit Juice (MFJ).

1.2.1 Specific Objectives

1- To measure the antioxidant capacity of MFJ through in vitro method by using common chemical analysis.

2- To analyze and identify the antioxidant compounds (free phenolic acids) in MFJ that can be used as marker compounds by using GC-MS.

3- To investigate the effects of MFJ supplementation on the safety parameters (clinical biochemistry profiles) and antioxidant enzymes defense system in normal rats (blood).

4- To investigate the effects of MFJ supplementation on the antioxidant enzymes defense system (blood and tissue) in aged rats.