Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/ijcas

Original Article

# Isolation and antimutagenic activity of some flavanone compounds from *Kaempferia rotunda*

*Sri Atun*, Retno Arianingrum, Eddy Sulistyowati, Nurfina Aznam*

*Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Yogyakarta State University, Karangmalang, Depok, Sleman, Yogyakarta 55281, Indonesia*

ARTICLE INFO

*Article history:*
Received 9 February 2013
Accepted 18 March 2013
Available online 24 March 2013

*Keywords:*
Antimutagenic
*Kaempferia rotunda*
Flavanone

ABSTRACT

*Background/Aims:* *Kaempferia rotunda* (Zingiberaceae), known as kunci pepet or kunir putih in Indonesia, has been traditionally used in abdominal pain, sputum laxative, wounds and diarrhea colic disorder. This study was conducted to isolate and to investigate antimutagenic activity of some flavanones from *K. rotunda*.

*Methods:* The milled dried rhizoma of *K. rotunda* (3 kg) was extracted exhaustively with methanol. The methanol extract was partitionated three times by *n*-hexane, chloroform, and ethyl acetate respectively. Each fraction was fractionated by vacuum liquid chromatography (VLC) and purified by column chromatography gravitation. Identification structures of all pure compounds were elucidated based on spectroscopic methods (UV, IR, and NMR) and compared to the spectroscopic previously reported data. Antimutagenic activity test was observed *in vivo* based on the number of micronucleated polychromatic cell erythrocytes (MNPCE) from male Balb-c mice (8–12 week) induced by cyclophosphamide.

*Results:* From the dried and milled rhizoma of *K. rotunda*, three known flavanones, namely 5-hydroxy-7-methoxyflavanone (1), 7-hydroxy-5-methoxyflavanone (2), and 5,7-dihydroxyflavanone (3) were isolated. The methanol extract and isolated flavanones from K. rotunda showed significant antimutagenic effect compared to control group.



## 1. Introduction

Cancer becomes a significant health problem in the world. According to WHO in 2000–2010 cancer ranks as the second cause of death worldwide after heart disease and in the 2030 is expected to increase and become the first ranks.[1] Cancer is the uncontrolled growth of cells, followed by cell invasion into the surrounding tissue and spread to other body parts. The main characteristic of cancer is a continuous cell proliferation, causing an imbalance between life and death cells.[2] Cancer is a multi-factorial, multi-stage and multimechanistic complex process with multiple risk factors that involve interplay between genetic and environmental components.[3] One of the factors that cause cancer is the mutation in a DNA gene. When a mutation is happen in the DNA, then the cancer may be very difficult to cure. The mutations in somatic cells are not only involved in the carcinogenesis process but also play a role in the pathogenesis of other chronic degenerative diseases, such

* *Corresponding author*. Fax: +62 (0) 274540713.
E-mail addresses: Atun_1210@yahoo.com, sriatun@uny.ac.id (S. Atun).
0976-1209/$ – see front matter 
http://dx.doi.org/10.1016/j.ijcas.2013.03.004

as atherosclerosis and heart diseases, which are the leading causes of death in the human population.[4] The mechanism of the mutation could be spontaneously and by the induction of some factors such as radiation, chemicals, and viruses. Mutagen is a substance that causes mutations, whereas compounds that can inhibit the mutation called antimutagenic.[5]

There is considerable evidence that the effects of mutagenic and carcinogenic agents can be altered by many dietary constituents or natural bioactive materials in many plant species. Investigations of antimutagenic potentials of herbal used in traditional medicine are generating great interest with the growing evidence of their safe consumption. Some herbs that have been studied as antimutagenic among others *Momordica charantia*,[5] ascorbic acid,[6] several compounds curcumin and its derivatives,[7] phenolic compounds such as ellagic acid,[8] and polyphenols in fruits, vegetables, and tea.[9]

*Kaempferia* genus is perennial member of the Zingiberaceae family and is cultivated in Indonesia and other parts of Southeast Asia. Number of studies has been conducted, providing information related to *Kaempferia* as chemopreventive agent. The methanol extract of *Kaempferia parviflora* showed a high cytotoxic activity against human cholangiocarcinoma (HuCCA-1 and RMCCA-1).[10] Some compounds such as panduratin A from *Kaempferia pandurata* showed high cytotoxic activity against human epidermis KB cancer cells,[11] showed high toxicity against human pancreatic cancer cells Panc-1.[12] The methanol extract of *K. parviflora* are also induced apoptosis of the cancer cells HL-60.[13] Previous studies showed that some compounds that have cytotoxic activity against cancer cells also showed antimutagenic properties.[4] This paper will report our investigation of some flavanones from *Kaempferia rotunda* and their antimutagenic activity.

## 2. Material and method

### 2.1. Apparatus

UV and IR spectra were measured with Varian Cary 100 Conc and Shimadzu 8300 FTIR, respectively. $^{1}H$ and $^{13}C$ NMR spectra were recorded with Jeol JNM A-5000 spectrometers, operating at 500.0 MHz ($^{1}H$) and 125.0 MHz ($^{13}C$) using residual and deuterated solvent peaks as internal standards. Evaporator Buchi Rotavapor R-114, vacuum liquid chromatography (VLC) was carried out using Si-gel Merck 60 GF254 (230–400 mesh), column chromatography using Si-gel Merck 60 (200–400 mesh), and TLC analysis on precoated Si gel plates Merck Kieselgel 60 F254 0.25 mm, 20 × 20 cm, water bath, shaker bath, microscup, camera, counter, deskglasser, eppendorf, object glass, and analytical balance.

### 2.2. Chemicals

Sodium carboxyl methyl cellulose (Na-CMC), cyclophosphamide monohydrate, physiological salin, xilol, Giemsa stain, were obtained from E. Merck in pure analytical grade. Several solvent such as chloroform, hexane, ethyl acetate, acetone, ethanol, and methanol.

### 2.3. Plant materials

Samples of the rizhoma of *K. rotunda* were collected in December 2010 from the Merapi Farma, Yogyakarta, Indonesia. The plant was identified by the staff at the Faculty Biology, Gadjah Mada University, Indonesia and a voucher specimen (KR-01–2012) was deposited at the Organic Laboratory, Yogyakarta State University, Indonesia.

### 2.4. Animal test

The experiment were carried out on adult male Balb-c mice (8–12 week) obtained from LPPT, Gadjah Mada University, Indonesia. All mice, 2–3 month old, weighed between 22, 5 and 27 g and were kept under constant environmental conditions with a 12: 12 light–dark cycle, at 23–25 °C room temperature. The animals were fed standard granulated chow (pelet 789) and had access to drinking water *ad libitum*. Animal experiments were done in accordance with Institutional Protocols of animal care. The mice were divided into ten groups consisting of six animal each. Group one served as normal control, group two was treated with cyclophosphamide doses at 50 mg/kg BW each day. Group three and four were treated with sample methanol extract *K. rotunda* doses at 300 and 600 mg/kg BW each day per *oral*, whereas group five until ten each group were treated with flavanone compounds (A, B, C) dose at 30 and 60 mg/kg BW daily per oral. Group three until ten at 30 min after treated with compounds followed cyclophosphamide doses at 50 mg/kg BW daily by *intravenal*. After 30 h treatment bone marrow from all the mice was collected respectively.

### 2.5. Antimutagenic assay

The antimutagenic assay of this experiment was determined by bone marrow micronucleus assay.[14] After 30 h the mice were anesthetized and the bone marrow was aspirated from femur and tibia into one ml of 1% physiological salin. The cell suspension was centrifuge (1000 rpm for 5 min) and the smears were prepared from the pellet on chemically cleaned glass slides and washed with ethanol absolute at 10 min, dried, and stained with Giemsa stain. To detect possible micronucleus, the portion of micronucleated polychromatic erythrocytes (MNPCE) in 1000 erythrocytes/mice was calculated by using a light microscope (1000× magnification). The frequency of MNPCE in individual mice was used as the experimental unit, with standard deviation based on difference among mice within the same group. The data from the micronucleus assay were statistically analyzed using Student's *t*-test, comparing the treated groups with control and significance level considered was $p < 0.05$. The percentage of antimutagenic activity calculated by the following formula:

$$\%\text{Activity} = \frac{(\text{mean CP} - (\text{mean S} + \text{mean N}))}{(\text{mean CP} - \text{mean N})} \times 100\%$$

Where CP = positive control group treated with cyclophosphamide; N = negative control group; S = group treated with methanol extracts or flavanones (A, B, or C)