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In Vitro Cytotoxic Activity of Two Malaysian Rainforest Plants on Colon Carcinoma Cell-Line

Alireza Nematollahi[1*], Noushin Aminimoghadamfarouj[1], Tracey D. Bradshaw[2], Christophe Wiart[3]

[1]School of pharmacy, Faculty of Science, Nottingham University, Malaysia
[2]Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK
[3]School of biomedical sciences, Faculty of Science, Nottingham University, Malaysia

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***Corresponding author:**

E-mail: khyx1ana@nottingham.edu.my
Phone: +60-10-4330493

ABSTRACT

Since the incidence rate of colon carcinoma is increasing significantly, the present study is undertaken to examine the in vitro cytotoxic activity of two unstudied Malaysian rainforest plants; *Uvaria grandiflora* Roxb (leaves and stem barks) and *Diospyros wallichii* King& Gamble (leaves and fruits), on the HTC116 colon carcinoma cell line. Different concentrations of the extracts obtained from each plant were subjected to cytotoxic investigation against HTC116 cell line by using MTT technique. The chloroform fraction of *Uvaria grandiflora* stem barks and the chloroform fraction of *Diospyros wallichii* fruits exhibit the highest growth inhibiting activities among all the tested samples with the GI_{50} value of 3.85 µg/ml and 4.58 µg/ml respectively. The obtained results warrant these plants for further bio-assay guided isolation in order to achieve novel leads.

INTRODUCTION

Today's developed countries are still struggling with the undeniable terminal disease named, cancer [1]. The occurrence of colon cancer, the fourth most common cause of cancer death, is increasing universally. Thus, it is logically and economically advised that serious investigations should be done in order to find novel colon cancer treatments [2]. The conventional treatment of cancer has faced new opportunities to get improved, through the new drug discoveries done on the medicinal plants and their secondary metabolites [3, 4]. Plants are almost considered to be the basis of traditional medicine system that has existed for thousands of years and is still providing mankind with new remedies [5]. Malaysia has enormous rainforests which elaborate broad arrays of natural medicinal compounds [6-8]. Annonaceae and Ebenaceae are two known medicinal plant families which have contributed in traditional treatments of different diseases for centuries in Malaysia [9-11]. There are lots of evidences introducing these mentioned medicinal plants to have strong cytotoxic and anticancer activities [12, 13]. The importance of Annonaceae is due to the existence of interesting chemical substances named acetogenins [9]. Likewise, the presence of naphthoquinones causes that Ebenaceae plants to manifest considerable cytotoxic activity [14, 15]. Therefore, the aim of the present investigation was to screen and evaluate the cytotoxic activity of different fractions obtained from *Uvaria grandiflora* Roxb, a rare Annonaceae, and *Diospyros wallichii* King& Gamble, a Malaysian endemic Ebenaceae, against the colon carcinoma cell line HTC116 by using MTT technique.

MATERIALS AND METHODS

Plant material

In December 2010 *Uvaria grandiflora* Roxb and *Diospyros wallichii* King& Gamble were collected from Perak forests in the Ipoh. The plant material was identified by the Forest research institute Malaysia.

Preparation of extracts

For each plant sample, plant materials were dried at room temperature in shade and then coarsely powdered. Dried samples were extracted with hexane, chloroform and ethanol by maceration. Dried fractions were obtained after removing the solvent by evaporation under reduced pressure and then were stored at -20 C^0 until tested.

Cell culture

colon carcinoma (HCT 116) cell line was sub cultivated twice weekly in RPMI 1640 supplemented with 10% fetal bovine serum and incubated at 37°C in an atmosphere of 95% air, 5% carbon dioxide. To minimize phenotypic drift, cell was maintained in culture for 4 months before being discarded and early passage cells resurrected from liquid nitrogen storage [16].

Growth Inhibition Assay

Cells were seeded into 96-well microtiter plates at a density of 5×10^3 per well and were allowed for 24 hours to adhere before drugs were introduced. Serial fractions dilutions were prepared in medium immediately before each assay. Viable cell masses at the

time of drug addition (time 0), and the following 72 hours of extract exposure were determined by cell-mediated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. MTT was added to each well and plates were incubated at 37°C for 4 hours in order to make the reduction of MTT by viable cell dehydrogenases into an insoluble formazan product possible. Well supernatants were aspirated and cellular formazan was solubilized by adding DMSO/glycine buffer (pH 10.5; 4:1). Cell growths as well as fractions activity were determined by measuring the absorbance at 555 nm using an Anthos Labtec systems plate reader [16].

RESULTS AND DISCUSSION

In order to evaluate the cytotoxic effect of the plant extracts that are used in Malaysian traditional medicine, a MTT assay with colon carcinoma cell line (HTC116) was performed. Table No.1 presents the cytotoxic activity results of the six different investigated fractions of *Uvaria grandiflora* and *Diospyros wallichii*. The maximum growth inhibiting activities belong to *Uvaria grandiflora* bark chloroform fraction and *Diospyros wallichii* fruit chloroform fraction with GI_{50} 3.85 and 4.58 µg/ml, respectively.

Table No.1: Growth Inhibiting activity (GI_{50}) of Malaysian plant fractions

Uvaria grandiflora fractions	GI_{50} (µg/ml)	*Diospyros wallichii* fractions	GI_{50} (µg/ml)
Hexane leaf fraction	>100	Hexane leaf fraction	>100
Chloroform leaf fraction	>100	Chloroform leaf fraction	5.80
Ethanol leaf fraction	>100	Ethanol leaf fraction	50.95
Hexane bark fraction	4.46	Hexane fruit fraction	NA*
Chloroform bark fraction	3.85	Chloroform fruit fraction	4.58
Ethanol bark fraction	71.53	Ethanol fruit fraction	67.7
Quercetin		21.47	
Negative Stantdard		Growth Complete	

* NA: Not Applicable

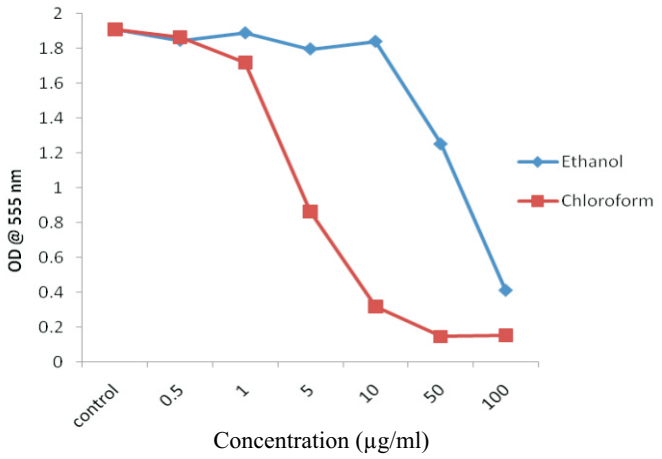

Fig.1: Effect of fruit extracts of *Diospyros wallichii* on HCT-116

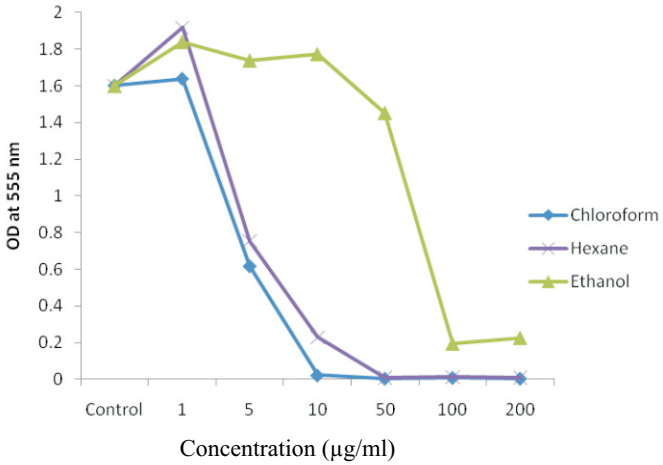

Fig. 2- Effect of bark extracts of *Uvaria grandiflora* on HCT-116

The figures 1 and 2 show the development of optical density and the concentration of the active fractions of each plant sample. The GI_{50} of the *Uvaria grandiflora* confirms that the bark chloroform fraction contains the interesting cytotoxic compounds. Based on the method of maceration and literature reviews it can be concluded that acetogenins or polyoxygenated cyclohexenes are possibly responsible for this activity [9]. The *Diospyros wallichii* GI_{50} values in the fruit chloroform and leave chloroform fractions may point out to naphthoquinones for the growth inhibition based on the maceration method and the partition coefficient of these chemical agents [10, 11].

CONCLUSION

These plants can be useful sources for drug development in treatment of colon cancer. To achieve this aim our research team is currently conducting further phytochemical investigations in order to isolate novel leads.

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