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In Vitro Antitumor Potential of *Canarium patentinervium* Miq

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Abstract: In the present study, six different extracts of *Canarium patentinervium* Miq. (Burseraceae) leaves and barks were screened for their *in vitro* antitumor activities, among the different extracts tested, the ethanol and chloroform extract of barks showed significant antitumor activities with GI_{50} values of 23.44 µg/ml and 34.40 µg/ml. The most susceptible cell lines were found to be the breast cancer cell line, MDA 468. The results obtained suggest that further isolation studies can be performed on *Canarium patentinervium* Miq to identify the active constituents responsible for the antitumor activities.

Key words: Antitumor · *Canarium patentinervium* Miq. · MDA468 · Breast Cancer

INTRODUCTION

Natural products and their derivatives contribute more than half of all clinically administered drugs[1]. They possess a significant position in drugdiscovery for treatment of cancer and other infectious diseases [2]. Approximately 40% of newly approved drugs from 1983 to 1994 were of natural origin [3].

At present more than 450 different compounds have been isolated from active plants that have shown *in vitro* and/or *in vivo* antitumor activity. Virtually every major class of natural chemical compound is represented in the list of active constituents [4].

This clearly demonstrates that antineoplastic substances useful in effective management of human disease occur in plants. It is also known that nature is able to produce a wide variety of chemical entities of novel structure. Many of the new and novel compounds isolated from natural sources might otherwise have never been discovered, especially those of considerable complexity requiring the development of methods for the creation of new ring systems. Natural products appeared to be a promising source for new types of compounds with antitumor activity [5].

The present study was undertaken to screen the antitumor potential of *Canarium patentinervium* Miq. The plant is belongs to the family of Burseraceae best known for producing resins of economic, medicinal and cultural values such as frankincense, myrrh and copal [6]. This family consist of 18 genera and 700 species of trees [7]. In the Asia-Pacific region, some species of Burseraceae are used to treat haemorrhoids and to treat skin infections [8]. No cytotoxic studies have been reported on this species to date.

MATERIALS AND METHODS

Plant Material: The leaves and barks of *Canarium patentinervium* Miq. were collected from one individual tree in April 2010 from Bukit Putih, Selangor, Malaysia (3°5'24 "N 101°46'0"E). The plant was identified by Mr. Kamaruddin (Forest Research Institute of Malaysia). A herbarium sample (PID 251210-12) has been deposited in the Forest Research Institute of Malaysia. The leaves and barks were air dried and grinded into small particles using an industrial grinder.

Chemical and Reagents: Hexane and chloroform were purchased from Friendemann Schmidt Chemicals,

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methanol and ethanol 95% was purchased from Kollin[R] Chemicals, DMSO was from R& M Marketing, Essex UK. RPMI 1640medium, L-glutamine, glycine buffer and 10% fetal calf serum was purchased from Sigma Aldrich[R].

Extraction: Dried and grinded sample of leaves (2.8kg) and barks (1.7kg) were soaked in hexane with the ratio of 1:3 parts of sample to solvent for 2h in a 60°C water bath, then filtered and concentrated with a rotary evaporator (Buchi, R-200 Switzerland). This was repeated 3 times. Thereafter the leaves and barks were left to air dry completely for 3 days before repeating the whole process with chloroform and then ethanol respectively. The yield for the hexane, chloroform and ethanol extract of leaves were 1.25%, 1.11% and 6.45% respectively. The yields for the hexane, chloroform and ethanol extract of barks for were 1.04%, 0.4% and 2.61%. Crude extracts were kept at -20°C until further use.

***In vitro* MTT Assay:** Biological *in vitro* assay was determined as follows [9]:

Compounds were prepared as 400ug/ml top stock solutions, dissolved in DMSO and stored at 40°C, protected from light for a maximum period of 4 weeks. Human derived cell lines [HCT 116, colon cancer cell line and MCF-7(ER+), MDA 468 (ER-) breast carcinoma] were routinely cultivated at 37°C in an atmosphere of 5% CO2 in RPMI 1640medium supplemented with 2 mM L-glutamine and 10% fetal calf serum and sub cultured twice weekly to maintain continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of 3-5 × 10^3per well and allowed 24h to adhere before drugs were introduced (final concentration 200µg/ml to 1µg/ml). Serial drug dilutions were prepared in medium immediately prior to each assay. At the time of extract addition and following 72h exposure, MTT (3-(4,5-dimethylthazol-2-yl)-,5-diphenyl tetrazolium bromide) was added to each well. Incubation at 37°C for 4 h allowed reduction of MTT by viable cells to an insoluble formazan product. Well contents were aspirated and formazan solubilized by addition of DMSO: glycine buffer (pH 10.5) (4:1). Absorbance was read on an Anthos Labtec System plate reader at 550 nm as a measure of cell viability, thus cell growth or extract toxicity was determined.

Statistical Analysis: Optical density-concentration curves were calculated using the Microsoft Excelfor Windows and data were reported as mean and SD values obtained from a minimum of three determinations. Non linear curve was plotted.

RESULTS

Biological *In Vitro* Assay: MTT assay is dependent on the reduction of the tetrazolium salt MTT (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide) by the mitochondrial dehydrogenase of viable cells to

Table 1: Growth inhibition of human cancer cell lines by plant extracts

	GI_{50} µg/ml		
Cell lines/extract	Chloroform	Ethanol	Hexane
MDA 468			
L	>100	61.48	>100
B	23.44	34.40	>100
MCF-7			
L	>100	85.40	>100
B	64.50	39.06	>100
HCT 116			
L	>100	97.18	>100
B	62.46	46.53	>100

L-leaves, B-barks

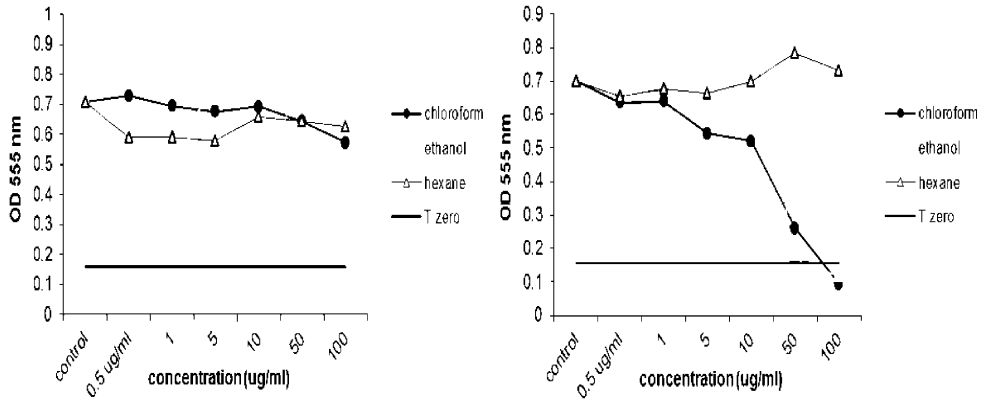

Fig. 1: Effects of extract of leaves and barks on MDA 468 growth

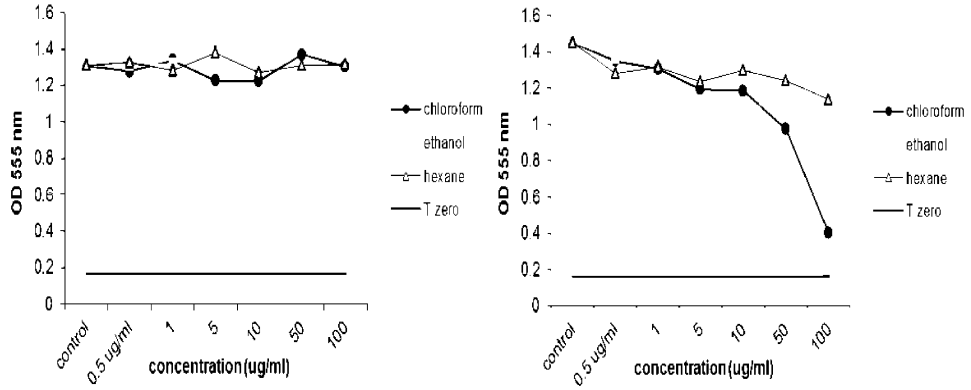

Fig. 2: Effects of extract of leaves and barks on MCF-7 growth

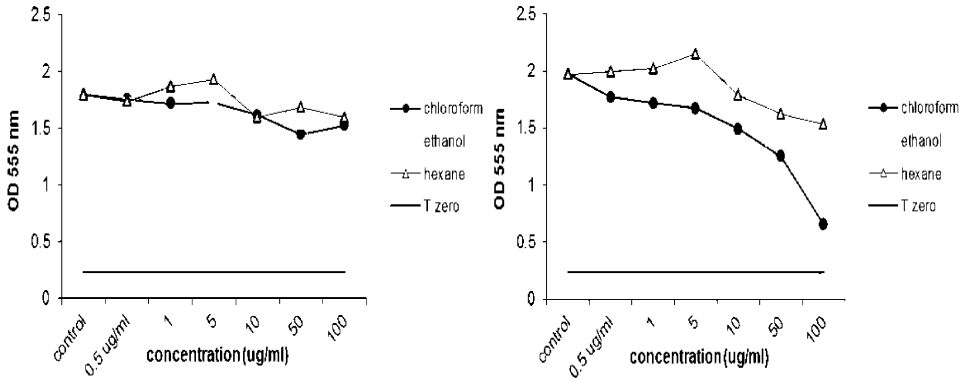

Fig. 3: Effects of extract of leaves and barks on HCT 116 growth

form a blue formazan product. The assay measures cell respiration and the amount of formazan produced is proportional to the number of living cells present in culture which will result in lower optical density (OD) [10]. A range of extracts were evaluated in MTT assays following a 3-day exposure against a panel of two human breast cancer cell lines, MCF-7 (ER+) and MDA 468 (ER-) and a colon cancer cell line, HCT 116. In general, the breast cancer cell lines were more sensitive to the extracts and the MDA 468 line was the most sensitive of the three lines (Table 1). The best growth inhibition was observed with the chloroform and ethanol extract of barks, GI_{50} values of 23.44µg/ml and 34.40µg/ml respectively (Figure 1-3).

DISCUSSION

Plant substances continue to serve as a wellspring of drugs for the world population and several plant-based drugs are in extensive clinical use [11]. In the present study, preliminary antitumor screening of six extracts of *Canarium patentinervium* Miq. showed that the chloroform and ethanol extract of the barks had promising growth inhibition on the breast cancer cell line MDA 468.

Results from our phytochemical analysis in previous studies revealed that the ethanol extracts barks of *Canariumpatentinervium*Miq. accumulate substantial amounts of flavonoids, steroids and tannins while the chloroform extract of barks tested positive for steroids which could be well correlated with the activities measured. We are in the process of conducting further work to isolate the antitumor constituents of the plant from the chloroform and ethanol extract of barks.

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