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RESEARCH ARTICLE

IN VITRO ANTICANCER EFFECT OF ARTABOTRYS CRASSIFOLIUS HOOK.F. & THOMSON AGAINST HUMAN CARCINOMA CELL LINES*Tan Kok Kwan¹, Bradshaw Tracey D.², Chu Jessica², Khoo Teng Jin¹, Wiart Christophe¹¹School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500, Semenyih, Selangor Darul Ehsan, Malaysia.²Centre for Biomolecular Sciences, School of Pharmacy, The University of Nottingham, University Park, Nottingham NG7 2RD, UK.*Corresponding author email: khyx1tkk@nottingham.edu.my, tkk20118@yahoo.com.sg**ABSTRACT**

The aim of the present study was to investigate the anticancer effects of crude extracts of *Artabotrys crassifolius* on the growth of human breast and colorectal carcinoma cell lines. The leaves and barks of *Artabotrys crassifolius* were extracted sequentially with hexane, chloroform and ethanol. The corresponding crude extracts obtained were then tested for their anticancer activity using MTT assay. Among the extracts studied, chloroform and hexane extracts of barks were found to possess the most potent anticancer activity which necessitates further isolation and characterisation of the bioactive compounds present in the respective extracts.

Keywords: Anticancer, *Artabotrys crassifolius*, Human carcinoma cell lines**INTRODUCTION**

Cancer is a group of diseases characterised by uncontrolled growth and spread of abnormal cells, which can lead to death if left untreated¹. The etiology of cancer can be associated with both external factors, including tobacco, infectious organisms, chemicals and radiation, as well as internal factors, such as inherited mutations, hormones, immune conditions and mutations occurring from metabolism². These causal factors may act synergistically or sequentially to initiate or promote carcinogenesis³.

Despite the considerable progress made over the last few decades in oncology research and treatment, cancer remains as one of the foremost causes of morbidity and mortality worldwide, with 12.7 million new cases and 7.6 million deaths in 2008⁴. More significantly, the most common cancer treatments are restricted to surgery, radiation and chemotherapy⁵, which are severely fraught with challenges concerned with adverse side effects of drugs⁶ due to their non-specific systemic distribution⁷, inadequate drug concentrations reaching the tumour⁸, intolerable toxicity⁹, and development of multiple drug resistance acquired upon repeated chemotherapeutic cycles¹⁰. Hence, there is clearly a need for novel chemotherapeutic agents with enhanced potency and specificity.

To the best of our knowledge, no detailed studies have been reported on the pharmacological properties of *Artabotrys crassifolius*. Therefore, the objective in this research was to investigate the anticancer effects of crude extracts of *Artabotrys crassifolius* against human breast and colorectal carcinoma cell lines.

MATERIALS AND METHODS**Collection and identification of plant material**

The leaves and barks of *Artabotrys crassifolius*, with the local name of *akar mempising*, were collected from Kuala Kangsar, Perak, Malaysia (4°46'N, 100°56'E) in March 2011. The plant was identified and authenticated by Mr. Kamarudin Saleh, Forest Biodiversity Division, Forest Research Institute Malaysia (FRIM). Voucher specimens were prepared and deposited in the Kepong Herbarium (KEP) of FRIM (PID 080311-05), and the School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus (UNMC 65) for future reference.

Preparation of plant material

After removal of extraneous matter, the freshly collected leaves and barks were air-dried in the shade at room temperature for at least 2 weeks. The dried leaves and barks were then finely pulverised by grinding prior to extraction. The pulverised leaves (1.30 kg) and barks (4.79 kg) were extracted sequentially with solvents of increasing polarity starting from hexane (Friendemann Schmidt, Australia), chloroform (Friendemann Schmidt, Australia) and 95% (v/v) of ethanol (John Kollin Chemicals, India). Each extraction was performed in triplicate at a solid-to-solvent ratio of 1:5 (w/v) in a 40°C water bath (Julabo, Germany) for three days. The respective extract was subsequently filtered through qualitative filter papers No. 1 (Whatman International Ltd., England) and the collected filtrate was concentrated to dryness under reduced pressure at 40°C using rotary evaporator (Buchi Labortechnik AG, Switzerland). Eventually, the dried extract obtained was weighed and stored at -20°C until further use. For stock solutions, each crude extract was dissolved in dimethyl sulphoxide (DMSO) (R & M Chemicals, UK) at a concentration of 100 mg/mL and stored at 4°C.

Cell lines and culture media

The cell lines used in the current study were derived from human carcinoma as shown in Table 1. Three human carcinoma cell lines were procured from the

Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham UK Campus.

Table 1: Types of human cell lines

Human cell line	
Origin	Designation
Breast carcinoma	MCF-7 (estrogen receptor-positive, ER+)
Breast carcinoma	MDA-468 (estrogen receptor-negative, ER-)
Colorectal carcinoma	HCT-116

Each cell line was routinely maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich, USA) supplemented with 2 mM of L-glutamine (Sigma-Aldrich, USA) and 10% (v/v) of foetal bovine serum (FBS) (Sigma-Aldrich, USA) at 37°C in a humidified 5% (v/v) of CO₂ incubator (Binder, Germany), and subcultured twice weekly to maintain continuous logarithmic growth.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The anticancer effects of crude extracts were investigated against human breast and colorectal carcinoma cell lines using MTT assay^{11,12}. Each cell line was seeded in 96-well microtiter plates (Jet Biofil, China) at a density of 5 × 10³ cells/well, and allowed to adhere for 24 h before crude extracts were introduced. Serial dilutions of crude extracts (final concentrations ranging from 6.25 µg/mL to 200 µg/mL) were prepared in medium immediately prior to assay. Viable cell masses at the time of crude extract addition (time zero) and following 72 h exposure were determined by cell-mediated reduction of MTT. A final concentration of 400 µg/mL of MTT (Sigma-Aldrich, USA) was added to each well, and plates were incubated at 37°C for 4 h to allow reduction of MTT by viable cells to an insoluble formazan product. The well supernatant was subsequently aspirated and the cellular formazan was solubilised by addition of DMSO (R & M Chemicals, UK) and glycine buffer (pH 10.5) (Sigma-Aldrich, USA) in a ratio of 4:1 (v/v). Eventually, the absorbance was read at 550 nm using an Anthos Labtec systems plate reader as a measure of cell viability.

Using the eight absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of crude extracts at the six concentration levels (Ti)], the percentage growth was calculated at each of the crude extract concentration levels¹³. Percentage growth inhibition was calculated as:

$$\frac{[(Ti - Tz)/(C - Tz)] \times 100}{Ti \geq Tz}$$

$$[(Ti - Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz$$

Three dose response parameters were calculated for each crude extract. Growth inhibition of 50% (GI₅₀) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which was the crude extract concentration resulting in a 50% reduction in the net cell growth during the incubation.

The crude extract concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The LC₅₀ (concentration of crude extract resulting in a 50% reduction of initial cells at the end of the treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested¹⁴.

Statistical analysis

Statistical analysis of experimental data was performed using Microsoft Office Excel data analysis software. Data were presented by descriptive analysis as mean and standard deviation of three independent experiments performed in triplicate.

RESULTS AND DISCUSSION

Plants have an almost unlimited capacity to synthesize diverse secondary metabolites that attract researchers and scientists in the quest for new chemotherapeutics¹⁵. The continuing exploration for novel chemical classes of anticancer agents in medicinal plants is one of the realistic and promising approaches for cancer prevention¹⁶. In the present study, MTT assay was conducted to investigate the anticancer effects of crude extracts on the growth of human breast and colorectal carcinoma cell lines.

This colourimetric assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate (MTT) into an insoluble, purple coloured formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the cell viability¹⁷.

According to the American National Cancer Institute (NCI), crude extracts could be considered as active for a GI₅₀ value of less than 20 µg/mL¹⁸. Based on the NCI criterion, chloroform extract of barks was highly active against all of the tested carcinoma cell lines with GI₅₀ values ranging from 4.23 µg/mL to 9.45 µg/mL (Figure 1–3). Furthermore, hexane extract of barks potently inhibited the growth of MDA-468 breast carcinoma cell line as well as HCT-116 colorectal carcinoma cell line, with respective GI₅₀ values of 6.10 µg/mL and 16.45 µg/mL. This may indicate that the non-polar active

principles present in the barks are responsible for the anticancer activity of this plant.

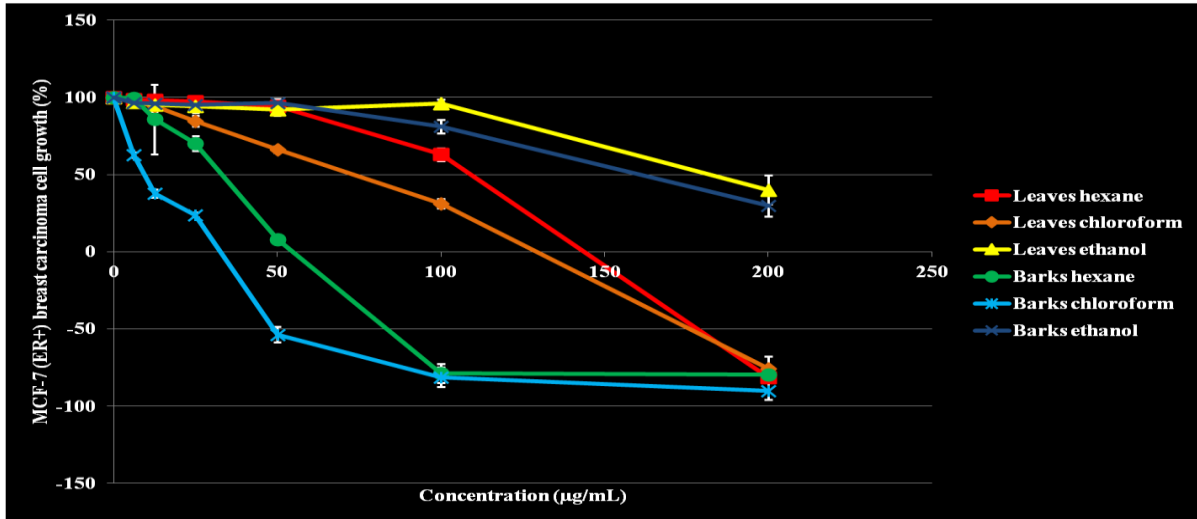


Figure 1: Effect of crude extracts of *Artabotrys crassifolius* on MCF-7 (ER+) breast carcinoma cell growth. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

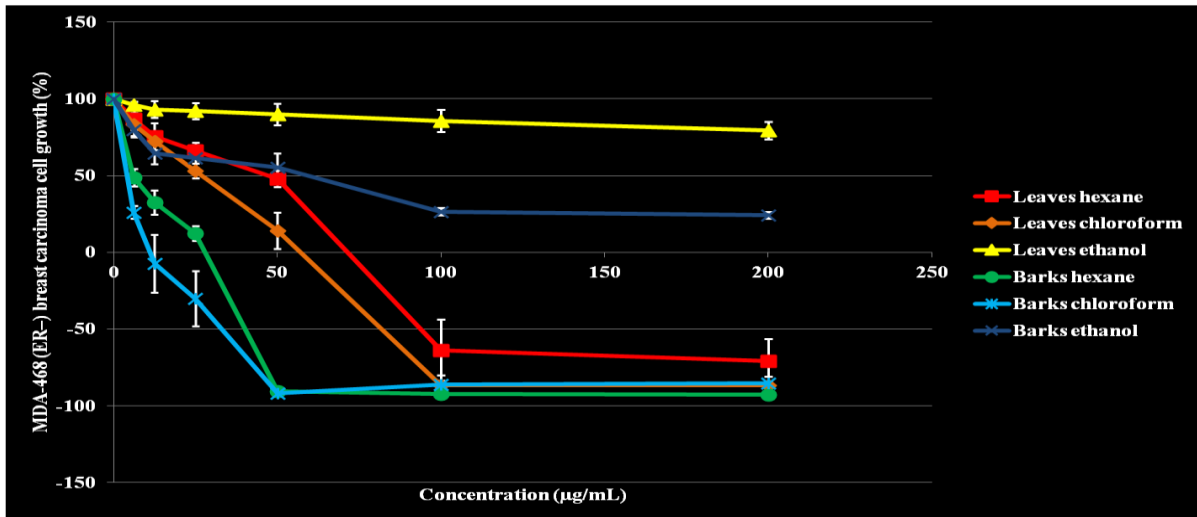


Figure 2: Effect of crude extracts of *Artabotrys crassifolius* on MDA-468 (ER-) breast carcinoma cell growth. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

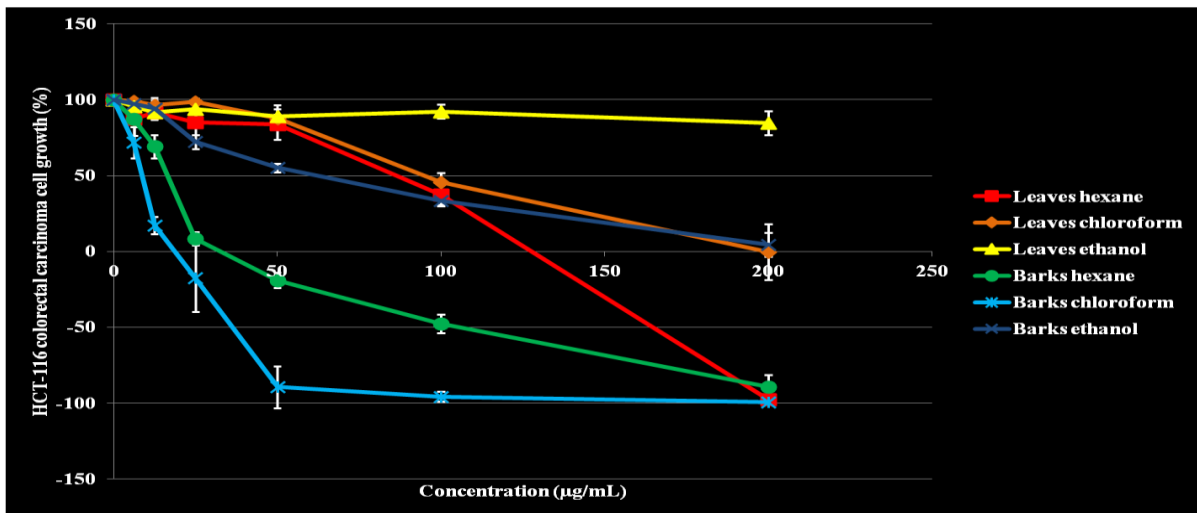


Figure 3: Effect of crude extracts of *Artabotrys crassifolius* on HCT-116 colorectal carcinoma cell growth. Results are expressed as mean ± standard deviation (error bar) of three independent experiments performed in triplicate, n = 9.

For better visualisation of the differences in achieving complete cell growth inhibition for all the crude extracts, TGI concentrations were expressed. In MCF-7 breast carcinoma cell line, chloroform and hexane extracts of barks were less potent as shown by their respective TGI values of 32.73 µg/mL and 54.63 µg/mL. In MDA-468 breast carcinoma cell line, chloroform extract of barks induced total growth inhibition at the lowest concentration of 11.11 µg/mL, whereas hexane extract of barks displayed a comparable TGI value of 28.01 µg/mL. In HCT-116 colorectal carcinoma cell line, chloroform extract of barks exhibited a 1.75-fold lower TGI concentration than that of hexane extract of barks. However, no total growth inhibition was obtained upon treatment with ethanol extract of leaves and barks.

At doses higher than the TGIs, net cell killing was observed in all of the tested carcinoma cell lines. Chloroform extract of barks demonstrated the highest cytotoxic action with LC₅₀ values ranging from 33.06 µg/mL to 48.84 µg/mL, while hexane extract of barks showed less pronounced cytotoxicity with LC₅₀ values ranging from 40.14 µg/mL to 105.61 µg/mL. Considering the TGI concentrations and the net cell killing achieved by crude extracts, MDA-468 breast carcinoma cell line was found to be the most sensitive cell line, followed by HCT-116 colorectal carcinoma cell

line, with MCF-7 breast carcinoma cell line being the least responsive to crude extracts.

With respect to the phytochemical screening of crude extracts¹⁹, the presence of alkaloids, cardiac glycosides and terpenoids in chloroform and hexane extracts of barks may explain their superior activity compared to the other crude extracts tested. This necessitates further isolation and characterisation of the potentially active principles from the respective crude extracts.

CONCLUSION

Examination of the *in vitro* anticancer effect of *Artabotrys crassifolius* revealed that chloroform and hexane extracts of barks may be a significant source of novel anticancer compounds in view of their promising activities particularly against MDA-468 breast carcinoma cell line. Therefore, further studies need to be carried out to isolate and characterise the bioactive compounds responsible for the observed anticancer properties of *Artabotrys crassifolius*.

ACKNOWLEDGMENT

The authors are grateful to Mr. Kamarudin Saleh for plant identification and authentication.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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