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Research Article

Cytotoxicity Effect of the Pericarp Extracts of *Garcinia forbesii* King on MCF-7 Breast Cancer and HepG2 Liver Cancer Cell Lines

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ABSTRACT

Background: The species from the genus Garcinia has long been used as traditional medicine for cancer treatment. **Objective**: To analyze the phytochemical contents and assess the cytotoxic effects of pericarp extracts of *Garcinia forbesii* King against MCF-7 breast cancer cells and HepG2 liver cancer cells. **Materials and Methods**: The phytochemical contents were analyzed using the thin-layer chromatography and the cytotoxic activity was assessed using the MTT assay method. **Results:** Phytochemical screening showed the presence of alkaloids, flavonoids, terpenoids and polyphenols. The cytotoxic activities of n-hexane, DCM and ethyl acetate extracts on MCF-7 cells were shown with IC_{50} 103.605±2.3410 µg/mL, 397.609±28.0534 µg/mL and 1,518.301±68.6379 µg/mL respectively, while the IC_{50} on HepG2 cells were 79.798±3.2261 µg/mL, 83.230±4.2557 µg/mL and 671.875±94.3338 µg/mL respectively. **Conclusion**: The n-hexane, DCM and ethyl acetate extracts from pericarps of G. *forbesii* King have cytotoxic activities against MCF-7 and HepG2 cancer cells, therefore, it has the potential to be developed as an anticancer.

Key words: *Garcinia forbesii* King., Cytotoxic, MCF-7, HepG2, Anticancer.

INTRODUCTION

According to the World Health Organization data in 2004, the top five cancer occurrences in the world were lung cancer, breast cancer, colon cancer, stomach cancer and liver cancer. The cancer incidence increased from 12.7 million cases in 2008 to 14.1 million cases in 2012. While the number of deaths from cancer increased from 7.5 million people in 2008 to 8.2 million in 2012. It is estimated that in 2030 the incidence of cancer may reach 26 million people and 17 million of them will die from cancer. In poor and developing countries, deaths from cancer increase faster[1]. In women, breast cancer has the highest percentage of new cases (43.3%), and the percentage of death is 12.9%[1]. In men, liver cancer incidences is on the second highest after lung cancer, with 14.5% deaths[2].

Many cancer drugs used today are derived from plants. Some of the results of previous studies found that natural anti-cancer compounds are from flavonoids, alkaloids and terpenoids[3]. Some natural compounds that have been widely used as cancer drugs are Taxol from *Taxus brevifolia*[4], and vincristine and vinblastine from *Vinca rosea*[5].

The plants from the genus Garcinia of the Clusiaceae tribe are widespread throughout the tropical and subtropical regions. The species from the genus Garcinia has long been used as traditional medicine in many countries, and contains many chemical compounds with many biological activities, including anticancer activity[6,7]. This species are known to contain many compounds such as flavonoids, benzophenone, lanostane, xanthon and terpenoids, which show anticancer activity. 7-epiclusianone, which is a benzophenone group is isolated from *Garcinia brasiliensis*[8]. Wallichinanes A-E, which is a group of lanostanes, is isolated from *Garcinia wallichii* Choisy[9], nujiangexathone A, which is a group of xanthones, is isolated from *Garcinia nujiangensis*[3].

Preliminary research on anticancer activity has been carried out on several Garcinia species. Many plants from Garcinia genus have cytotoxic effects, For example: the root of *Garcinia cowa*[10], the stem bark of *Garcinia ovalifolia*[11] and *Garcinia cylindrocarpa*[12], the leaves of *Garcinia nijuangensis*[3], the fruits of *Garcinia wallichii*[9], the pericarps of *Garcinia mangostana*[13], *Garcinia brasiliensis*[8] and *Garcinia dulcis*[7]. The fruits, flesh and seeds of the *Garcinia dulcis* are also shown to contain active compounds that are apoptotic to HepG2 liver cell lines[7]. The pericarps of *Garcinia mangostana* have cytotoxic effect on nasopharyngeal cancer cell lines CNE1, CNE2, SUNE1 and HONE1; lung cancer cell lines A549 and GLC82; MCF-7 breast cancer cell lines; and Bel-74021[14] and Hep-G2 liver cancer cell lines[15].

Garcinia forbesii King is one of the species that is widely spread on the island of Kalimantan (Indonesia) and parts of Malaysia[16]. In Banjar, South Kalimantan, this plant is known by its local name Mundar. The phytochemical isolation of this plant extract has found xanthones, 1,3,7-trihydroxy-2- (3-methylbut-2-enyl) -xanthone and forbexanthone compounds[17]. Another study has isolated rubraxanthone, which has antibacterial effect, from the stem bark of this plant[18].

No previous publications of study results regarding anticancer activity of the pericarp of G. *forbesii* King have been found. Therefore, in this study we assessed the cytotoxic effects of n-hexane, DCM, ethyl acetate,

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n-butanol and water extracts from the pericarps of G. *forbesii* King on MCF-7 breast cancer cells and HepG2 liver cancer cells.

MATERIALS AND METHODS

Collection of plant materials

The pericarps of G. *forbesii* King were collected in December 2016 in Banjar, South Kalimantan. This plant was identified by the botanist of the UPT Plant Conservation Center, Purwodadi Botanical Garden, LIPI, East Java.

Extraction

The pericarps of G. *forbesii* King were cleaned, dried, and then ground into fine powder. The pericarp powder was extracted by maceration using n-hexane solvent for 1x24 hours and the filtrate was collected. The maceration was repeated three times. All the filtrate was collected and evaporated with a rotary evaporator to obtain a n-hexane dry extract.

The residue obtained from the rest of maceration with n-hexane was dried and macerated again using DCM solvent for 1x24 hours, and this process was repeated three times. The collected filtrate was evaporated with a rotary evaporator until the DCM extract was obtained. Further, the maceration was carried out in the same manner successively using ethyl acetate, n-butanol, and water solvents. The extracts of n-hexane, DCM, ethyl acetate, n-butanol and water were analyzed for their phytochemical contents, and their cytotoxic activities were assessed against MCF-7 breast cancer cells and HepG2 liver cancer cells.

Thin Layer Chromatography (TLC) analysis[19-21]

The TLC was performed using Kiesel Gel GF254 as the stationary phase. The concentration of sample was 1% w/v.

Flavonoids screening was conducted using the n-butanol:glacial acetic acid:water (4:1:5) as the mobile phase, and the ammonia vapor as the appearance spots.

Polyphenols screening was conducted using the chloroform:ethyl acetate:formic acid (1:18:1) as the mobile phase, and $FeCl_3$ as the appearance spots.

Terpenoids screening was conducted using the n-heksana:ethyl acetate (4:1) as the mobile phase, and anisaldehyde sulfuric acid as the appearance spots.

Alkaloids screening was conducted using the chloroform:ethyl acetate (1:1) as the mobile phase and the Dragendorf reagent as the appearance spots.

Cell culture[21]

The cancer cells used were MCF-7 cell line and HepG2 cell line obtained from the Parasitology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. The cells were grown in RPMI media. After the cell condition reached 80% confluent, the cells were harvested according to the harvest protocol. The cells were suspended in RPMI to a concentration of $5x10^3$ cells/100 µL. The cell suspension was inserted into the wells, each 100 µL for each well. Every time the cell suspension was inserted into 12 wells, the cells were resuspended again to ensure homogeneity. The cells were incubated in the incubator overnight to allow for cell recovery after harvesting. The plate containing cells was taken from the incubator, and the media was discarded by turning the plate 180°C above the dump. The plate was pressed gently over a tissue paper to drain the remaining liquid. 100 µL PBS was put into all the wells filled with cells, and then the PBS was discarded by turning the plate. The remaining liquid was drained with tissue paper.

Preparation of extracts solutions for cytotoxicity assay[22,23]

A total of 10 mg of each extract was dissolved in 100 µL DMSO, and a stock solution of $1x10^5$ ppm was obtained. From the stock solution, sample solutions were made with a concentration of 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm and 15,625 ppm using culture media as the diluent.

Cytotoxicity assay[21]

Each sample concentration was put into a well containing cancer cells, three wells for each concentration (triplo). The plate was incubated in an incubator for 24 hours. After 24 hours the cell media was removed, washed using PBS 1x, and 100 µL MTT reagent was added to each well, including media control. Cells were re-incubated for 2-4 hours in an incubator until the formazan was formed. Cell conditions were examined with an inverted microscope. After the formazan has clearly formed, a 10% SDS stopper in 0.1 N HCl was added. The plate was wrapped in aluminum foil and incubated in a dark room temperature overnight. After the incubation, the plates was inserted into the ELISA reader, and the absorbance of each well was analyzed with λ = 550-600 nm. Absorbance vs. concentration graphs were made to illustrate the profile of living cells, and the percentage of living cells and IC50 value were calculated using linear regression.

RESULTS

Chemical contents of the extracts

The results of TLC analysis of the extracts are shown in Table 1.

Cytotoxic activity

The cytotoxic activity of each extract against MCF-7 and HepG2 cancer cells expressed by the IC50 values is as shown in Table 2.

DISCUSSION

The results of TLC analysis of several extracts of G. *forbesii* King pericarps showed that the n-hexane extract contained flavonoids and terpenoids which quantities were larger than the other extracts. The contents of flavonoids and terpenoids decreased with increasing polarity of the solvents in the successive in G. *forbesii* king pericarps. The content of alkaloids was very small in quantity, and it was only detected in n-hexane and DCM extracts, whereas in other extracts there was no detectable alkaloids. Polyphenols were present in relatively

Table 1: The chemical contents of *Garcinia forbesii* King. Extracts.

Extract	Flavonoids	Polyphenols	Terpenoids	Alkaloids
n-hexane	+++	+	+++	+
DCM	++	+	++	+
Ethyl acetate	++	++	+	-
n-butanol	+	+	+	-
Water	+	+	+	-

Note: -not detectable, +low intensity, ++medium intensity, +++high intensity

Table 2: Cytotoxic activities of *Garcinia forbesii* King. Extracts.

Extract	IC_{50} values on MCF-7 (µg/mL)	IC_{50} values on HepG2 (µg/mL)
n-hexane	103.605 ± 2.3410	83.230 ± 4.2557
DCM	397.609 ± 28.0534	79,798 ± 1.2261
Ethyl acetate	1,518.301 ± 68.6379	671,875 ± 94.3338
n-butanol	NC	NC
Water	NC	NC

NC: Not Countable

small amounts in almost all non-polar and polar extracts, except in ethyl acetate extract, where they were detected in slightly more amount.

The results of phytochemical screening from the extracts of G. *forbesii* King pericarps are in line with the results of previous studies on chemical properties in plants from the same genus. For example, the flesh of G. *dulcis* contains large amounts of flavonoids in the form of xanthones [7]. The bark of G. *cylindrocarpa* contains xanthones which belong to the flavonoid group[12]. Flavonoids and polyphenols are contained in pericarps of G. *Mangostana*[14,15,24]. The stem bark of G. *cola* contains alkaloids, flavonoids and terpenoids[25]. The fruits of G. *scomburgkiana* contains biflavonoids and flavones[26]. Xanthone flavonoids are contained in the roots of G. *ovalifolia*[11]. In G. *forbesii* itself, the bark contains forbexanthone and rubraxanthone[17,18].

In vitro anticancer potential is indicated by IC_{50} values. Smaller IC_{50} value indicates that the drug or test material is more potent as an anticancer. In this study, the anticancer potential assessed using the MTT essay method showed that the n-hexane extract (IC_{50} 103.605 ± 2.3410 µg/mL) had a higher anticancer potential compared to the DCM extract (IC_{50} 397.609 ± 28.0534 µg/mL), and the ethyl acetate extract (IC_{50} 1,518.301 ± 68.6379 µg/mL) against MCF-7 breast cancer cells. For HepG2 liver cancer cells, the DCM extract (IC_{50} 79.798 ± 1.2261 µg/mL) showed a higher anticancer potential compared to the n-hexane extract (IC_{50} 83.230 ± 4.2557 µg/mL), and the ethyl acetate extract (IC_{50} 671.875 ± 94.3338 µg/mL). The n-butanol and water extracts did not show any anticancer potential against the two cell lines, as indicated by they very high IC_{50} values.

The results shown by the n-hexane and DCM extracts indicate that the compounds that actively kill or inhibit the growth of HepG2 liver cancer cells and MCF-7 breast cancer cells may be non-polar and semi-polar compounds, especially flavonoids and terpenoids. This is in line with some of the results of previous studies. Sukandar *et al.*[12] found several flavonoid compounds such as cylindroxanthones A-C isolated from G *cylindrocarpa* which had cytotoxic activity against MCF-7 cancer cells with IC_{50} of 98.54 µM, 168.53 µM and 59.05 µM, respectively, and against HepG2 cancer cells with IC_{50} of 10.41 µM, 59.53 µM, and 37.90 µM, respectively. Hongthong *et al.*[9] found several terpenoid compounds namely wallichinanes A-E isolated from G. *wallichii* had cytotoxic activity against MCF-7 with IC_{50} of 6.19 µM for wallichinanes D compounds, and >50 µM for other compounds.

CONCLUSION

The pericarps of G. *forbesii* King contains flavonoid, polyphenol, terpenoid and alkaloid compounds.

The decreasing order of potential cytotoxic activity against MCF-7 cancer cells is n-hexane, DCM and ethyl acetate extracts, whereas for the cytotoxic activity against HepG2 cancer cells, the decreasing order was DCM, n-hexane, and ethyl acetate extracts. The n-butanol and water extracts did not show potential cytotoxic activity againts the two cells.

Extracts of n-hexane, DCM and ethyl acetate from pericarps of G. *forbesii* King have cytotoxic activity against MCF-7 and HepG2 cancer cells, therefore it has the potential to be developed as an anticancer.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

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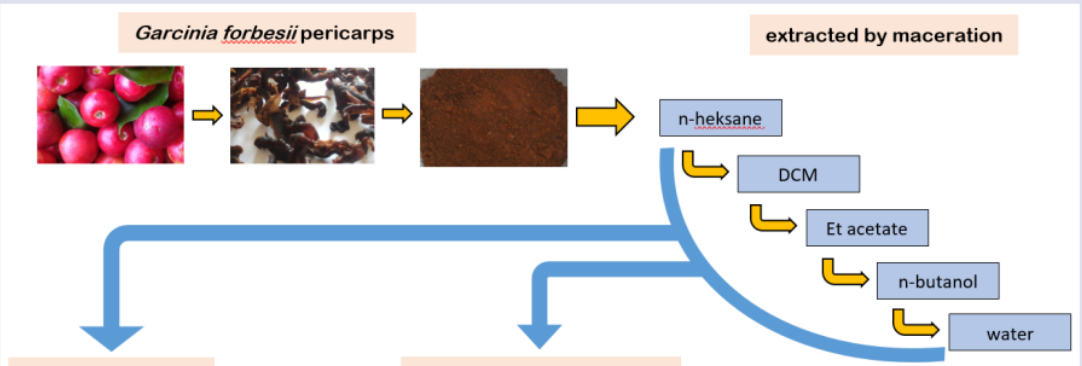
GRAPHICAL ABSTRACT



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Ethyl acetate	1,518.301±68.6379	671,875±94.3338
n-butanol	NC	NC
Water	NC	NC

Extract	Flavonoids	Polyphenols	Terpenoids	Alkaloids
n-heksane	+++	+	+++	+
DCM	++	+	++	+
Et acetate	++	++	+	-
n-butanol	+	+	+	-
Water	+	+	+	-

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