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Submission date: 24-Feb-2023 10:49AM (UTC+0700)
Submission ID: 2021768664
File name: siding_Internasional_-_Thin_Layer_Chromatography_Analysis_Of.pdf (495.71K)
Word count: 2098
Character count: 11290

THIN LAYER CHROMATOGRAPHY ANALYSIS OF ETHANOL EXTRACT OF *Garcinia forbesii* King. AND ITS CYTOTOXIC EFFECT ON MCF-7 BREAST CANCER AND HepG2 LIVER CANCER CELL LINES

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Thin Layer Chromatography (TLC) analysis of ethanol extract of *Garcinia forbesii* King. and its cytotoxic effect on MCF-7 breast cancer and HepG2 liver cancer cell lines has been conducted. Parts of the plant examined were leaves, stem bark, wood, and pericarp. The extraction was processed by maceration method, while the determination of the compound was carried out using various color reagents on TLC plate. The cytotoxic effect was carried out using MTT assay. TLC analysis showed that ethanol extract of the leaves, stem bark, and wood contains polyphenols, flavonoids, and terpenoids; while ethanol extract of the pericarp contains alkaloids, flavonoids and terpenoids. MTT assay showed the IC_{50} values of ethanol extract of the leaves, stem bark, wood, and pericarp in MCF-7 cell line were 197.92 µg/mL, 437.21 µg/mL, 164.11 µg/mL, and 60.11 µg/mL, respectively; while the IC_{50} values in HepG2 cell line were 88.58 µg/mL, 1403.75 µg/mL, 111.71 µg/mL, and 47.10 µg/mL.

Keywords: TLC, *Garcinia forbesii* King., cytotoxicity

INTRODUCTION

Many plants from *Gracinia* genus, belonging to the family Clusiaceae have cytotoxic effect. For example, the root of *Garcinia cowa*(1), stem bark of *Garcinia ovalifolia*(2) and *Garcinia cylindrocarpa*(3), leaves of *Garcinia nijuangensis*(4), fruits of *Garcinia wallichii*(5), pericarps of *Garcinia mangostana*(6), *Garcinia brasiliensis*(7) and *Garcinia dulcis*(8).

Various types of compounds such as flavonoids, benzophenones, lanostanes, xanthones and terpenoids were isolated from the Garcinia genus.

The 7-epiclusianone compound of benzophenone isolated from the pericarps of *Garcinia brasiliensis* inhibits the growth of gliablastoma cell lines (U138MG and U251MG)(7). The compound also decreases the growth of lung cancer cell lines (A549)(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[8]. The pericarps of *Garcinia mangostana* has 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-7402 liver cancer cell lines[9] and Hep-G2 liver cancer cell lines[10].

Garcinia forbesii King is the plants of the genus Garcinia that grows in Indonesia. This plants of Clusiaceae genus is fruit plant that is the same family with mangosteen. It has pink to dark red skin, with white flesh that is very similar to mangosteen flesh. This plant is widely grown on the Borneo island and parts of Malaysia[11]. The studies that have been carried out on this plant are has been isolated xanthone, 1,3,7-trihydroxy-2- (3-methylbut-2-enyl)-xanthone and forbexanthone compounds[12]. Another study has been isolated rubraxanthone from the stem bark of this plant which has antibacterial effects[13].

Therefore, we aim to perform thin layer chromatography (TLC) analysis to determination of compounds content, and cytotoxic investigation to delve the effects on cancer cell lines.

RESEARCH METHODS

Collection of plant material

The leaves, stem bark, wood, and pericarp of *Garcinia forbesii* King, was collected in Banjar, South Kalimantan in December 2016 and identified by UPT Balai Konservasi Tumbuhan, Kebun Raya Purwodadi, LIPI, East Java.

Extraction

The leaves, stem bark, wood and pericarps was washed, chopped into small pieces and dried under shade. The air-dried samples (100 g) was extracted with 100 mL ethanol by maceration at room temperature for three days. The filtrate solution was then subjected to vacuum rotary evaporator at 40ºC to remove ethanol residue[14].

Thin Layer Chromatography (TLC) analysis[15]

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

Flavonoids screening using the n-butanol:glacial acetic acid:water (4:1:5) as a mobile phase, and used the ammonia vapor or borate citrate as the appearance spots.

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

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

Alkaloids screening using the chloroform:ethyl acetate (1:1), and used dragendorf reagent as the appearance spots.

Cytotoxicity assay

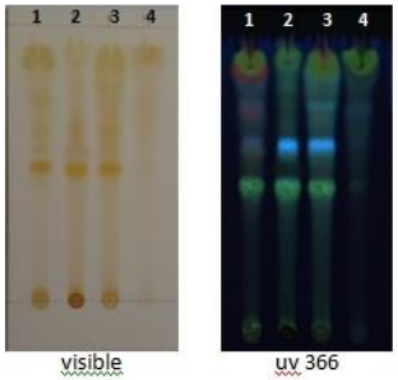
The ethanol extract of the leaves, stem bark, wood and pericarps were applied to in vitro cytotoxic evaluation against MCF-7 breast cancer and HepG2 liver cancer cell lines by using MTT colorometric method. The cancer cell lines suspended in 100 µg/wells of medium containing 10% fetal calf serum were seeded onto a 96-well culture plate and incubated for 24 h in a 5% CO_2 incubator at 37ºC. After 24 h, various concentrations of samples were added and these were then incubated for 48 h under the above conditions. Then, 10 μL of MTT reagent (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well and left in

incubator for 4 h. Subsequently, 100 μL of solubilization solution (DMSO) was added to each well and the optical density was detected using ELISA reader at 550 nm. The 50% inhibition concentration (IC_{50} value) was determined by linier regression[16].

RESULTS AND DISCUSSION

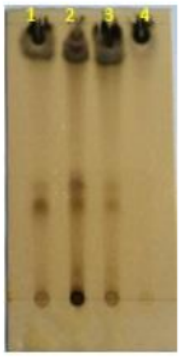
Thin Layer Chromatography (TLC) analysis

TLC analysis showed the presence of a class of compounds found in parts of *Garcinia forbesii* King. The results of flavonoid screening showed yellow spot after use ammonia vapor as reagent on ethanol extract of leaves, stem bark, wood and pericarps (Picture 1).



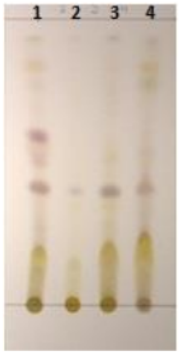
Picture 1. Flavonoid TLC analysis of ethanol extract of leaves[1], stem bark[2], wood[3] and pericarp[4].

The result of polyphenols screening showed black spot after use $FeCl_3$ as reagent on ethanol extract of leaves, stem bark and wood (Picture 2).



Picture 2. Polyphenols TLC analysis of ethanol extract of leaves[1], stem bark[2], wood[3] and pericarp[4].

The result of terpenoids screening showed red purple spot after use anisaldehyde sulfuric acid as reagent on ethanol extract of leaves, stem bark, wood and pericarps (Picture 3).



Picture 3. Terpeoids TLC analysis of ethanol extract of leaves[1], stem bark[2], wood[3] and pericarp[4].

The result of alkaloids screening showed orange spot after use dragendorf as reagent on ethanol extract of pericarps (Picture 4).

Picture 4. Alkaloids TLC analysis of ethanol extract of leaves[1], stem bark[2], wood[3] and pericarp[4].

Cytotoxic Effect

The incubation of ethanol extract of *Garcinia forbesii* King leaves, stem bark, wood and pericarp with MCF-7 breast cancer and HepG2 liver cancer cell lines induces cytotoxicity with IC_{50} values of the leaves, stem bark, wood, and pericarp in MCF-7 cell line were 197.92 µg/mL, 437.21 µg/mL, 164.11 µg/mL, and 60.11 µg/mL, respectively; while the IC_{50} values in HepG2 cell line were 88.58 µg/mL, 1403.75 µg/mL, 111.71 µg/mL, and 47.10 µg/mL (Table 1). The pericarps extract against MCF-7 and HepG2 cell line showed the lowest IC_{50} value.

Table 1. IC_{50} value of samples tested against MCF-7 and HepG2 cell lines by MTT assay

Ethanol extract	IC_{50} value (µg/mL)	
	MCF-7	HepG2
Leaves	197.92	88.58
Stem bark	437.21	1403.75
Wood	164.11	111.71
pericarps	60.11	47.10

Various cytotoxic compounds are found in the Garcinia genus, such as xanthones[1,4,17], terpenoids[5], benzophenones[7], alkaloids[18] and polyphenols[19]. Some parts of the plant of the Garcinia genus also have cytotoxic effects, such as fruit[8], pericarp[6], flesh[8], leaves[4], stem bark[3] and roots[1].

The ethanol extract of the pericarp of Garcinia forbesii King showed the best IC_{50} value compared to the leaves, stem bark and wood against MCF-7 and HepG2 cell lines. While the results of TLC analysis, the pericarp contains alkaloids, flavonoids and terpenoids, so it is likely one of the class of compounds have cytotoxic effects.

Some pericarps from Garcinia genus does have cytotoxic effect, such as G. *mangostana*[6], G. *wallichii*[5], G. *brasiliensis*[7], G. *Morella*[20], G. *dulcis*[8] and G. *schomburgkiana*[21]. This suggests that the similarity of taxonomy and similarity parts of the plant can provide the similarity of class of compounds and similarity effects.

CONCLUSION

TLC analysis showed that ethanol extract of the leaves, stem bark, and wood contains polyphenols, flavonoids, and terpenoids; while ethanol extract of the pericarp contains alkaloids, flavonoids and terpenoids. The ethanol extract of the pericarp of Garcinia forbesii King showed the best activity compared to the leaves, stem bark and wood against MCF-7 and HepG2 cell lines with IC_{50} value 88.58 µg/mL and 47.10 µg/mL.

TLC analysis is recommended to some extract of the pericarp of *Garcinia forbesii* King by using various organic solvent with various ranges of polarity, and its cytotoxic effects on various cancer cells.

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