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## RESEARCH ARTICLE

**ANTIOXIDANT AND CYTOTOXIC EFFECTS OF CURCUMA MANGGA AND BOSENBERGIA ROTUNDA ETHANOLIC EXTRACTS ON MCF-7 CANCER CELL LINES**Tamil C.M. Sundram<sup>1\*</sup>, Mohd Hazim Bin Zakaria<sup>1</sup> and Mohd Hamzah Bin Mohd Nasir<sup>2</sup><sup>1</sup>Department of Plant Science, Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia<sup>2</sup>Central Research and Animal Facility Management (CREAM), Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia\*Corresponding author e-mail: [tamil@ium.edu.my](mailto:tamil@ium.edu.my)

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## ARTICLE DETAILS

## ABSTRACT

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*Curcuma mangga* and *Bosenbergia rotunda* which belongs to Zingiberaceae family are largely distributed in South East Asian countries including Malaysia. These plants have been traditionally used for treatment of various diseases. In this research we focused on determining the antioxidant and cytotoxic activity of *C. mangga* and *B. rotunda* ethanolic extracts. Initially, in this study, ethanolic extracts from rhizomes of *C. mangga* and *B. rotunda* were obtained through Soxhlet extraction method. 12.6% and 3.5% of crude extract were obtained from *C. mangga* and *B. rotunda* respectively. For MTT assay, the IC<sub>50</sub> values are 207 µg/ml and 31.96 µg/ml for *C. mangga* and *B. rotunda* respectively. Meanwhile for the antioxidant activity using DPPH assay, *C. mangga* showed higher antioxidant activity with IC<sub>50</sub> values of 1073 µg/ml compared to *B. rotunda* with IC<sub>50</sub> values of 578 µg/ml. It can be concluded both extracts showed low inhibitory effect on MCF-7 cancer cells growth and DPPH free radical activity.

## KEYWORDS

*Bosenbergia rotunda*, antioxidant, *C. mangga*, *B. rotunda* ethanolic extracts.

## 1. INTRODUCTION

Southeast Asia (SEA) is well known for its diversity of flora and fauna. Different varieties of herbal plant species found in this part of world are used as remedies in medicines. Zingiberaceae is a family of herbal plants made up of about 50 genera with a total of about 1600 known species of aromatic perennial herbs with tuberous rhizomes. *Curcuma mangga* (Temu pauh) and *Bosenbergia rotunda* (Temu kunci) are two plants of this family well-known for their medicinal values [1].

*Bosenbergia rotunda* is a perennial herb plant with fleshy, short and slender rhizomes with a characteristic aroma and low pungent taste [2]. It is used as spices in culinary as well as ingredients in traditional medicine. In ethnomedicine, diseases such as inflammation, aphthous ulcer, dry mouth, stomach discomfort, dysentery, leukorrhea, rheumatism, muscular pains and kidney disorders have been successfully treated by the old folks using *B. rotunda* rhizome extract [1]. Recently, antimutagenic, antibacterial, antifungal, analgesic, antipyretic, antispasmodic, anti-inflammatory and pesticide properties of this plant extract have been reported [2].

Meanwhile *Curcuma mangga* is a rhizomatous herb with distinct identity of raw mango flavour combined with that of ginger flavour. *C. mangga* is also used as food seasoning and as therapy for stomach aches and fever. The extracts of the rhizomes are also used as alternative treatment for liver cancer and hepatitis B [3]. Some researcher found that *C. mangga* contains antioxidant, antitumour, antifungal and anti-allergic properties [4].

Breast cancer has been the most common cancer among women, with MCF-7 cell lines identified as the main breast cancer causing cell lines [5].

Currently, chemotherapy is being the common clinical practice used to kill breast cancer cells. However, this method which uses chemoradiation to kill the dividing cancer cells causes post treatment sickness such as numbness, fatigue and nausea to the patient. Moreover, the cost of the treatment is quite expensive and the survival rate after the treatment is still low [6]. Therefore, various alternative methods are currently being experimented in treating cancer.

Bioactive compounds from herbal plants are one, out of many alternatives that are continuously being tested as potential medicine for treatments of cancer. According to a study, plants from Zingiberaceae family had been reported to contain bioactive compounds with antioxidant and anti-cancer properties [6]. Free radical scavenging compounds are commonly found in most plant of this family. These compounds with antioxidant activity have the potential to slow down the cancer cells division process [1]. One success story of *B. rotunda* has been inhibition of hepatocellular and lung carcinoma cell growth by its crude extract [2]. Meanwhile, *C. mangga* extracts shows anti telomerase activity that is useful in inhibiting cancer cell growth [7]. Thus, in this study we aimed to test the antioxidant and cytotoxic activity of *C. mangga* and *B. rotunda* ethanolic extract against MCF-7 cell lines.

## 2. MATERIALS AND METHODS

## 2.1 Collection of Plant Materials

The fresh samples of *C. mangga* and *B. rotunda* were obtained from Greenhouse and Nursery Complex, IIUM Kuantan and also bought from Pasar Besar UTC, Kuantan. The plants were washed and rinsed with distilled water to remove the sand particles.

## 2.2 Preparation of crude extract

About 1000g of *C. mangga* and 1000g of *B. rotunda* were cut into thin pieces of approximately 3-5cm and freeze dried. Dry weight determined? After 2 days of freeze drying, the samples were extracted using Soxhlet extraction method with 99% ethanol (250mL) at the temperature of 27°C. The extraction was repeated until the solvent was colourless. The ethanolic extract was evaporated using rotary evaporator at 37 °C under the pressure of 0.8MPa in order to obtain the crude extract. The crude extracts were collected in separate beakers and weighed. The collected crude extracts were stored at 4°C temperature.

## 2.3 Antioxidant Assay

### 2.3.1 Preparation of Ascorbic Acid Standard

Ascorbic acid was used as positive control. 200 mg ascorbic acid was dissolved in 2 mL of 99% ethanol. The solution was diluted through serial dilution until it reaches the final concentration of 360 to 11.25µg/mL.

### 2.3.2 Determination of Antioxidant Activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Test

Crude extracts of plant samples were tested by DPPH assay to determine the antioxidant properties. Stock solution of 100 mg/ml concentration was prepared by dissolving 300 mg of each plant crude extract into 3 mL of 99% ethanol. Then, DPPH solution was prepared by dissolving 0.0019 g DPPH with 50 mL of 99% ethanol. The solution of DPPH was freshly prepared for every new test. The crude extract solution was diluted by serial dilution method to seven different concentrations (200 to 3.13 µg/mL). The test was done in a flat bottom 96 wells plate. The test was done by adding 100 µL of crude extract prepared at different concentration (including positive control) with 100 µL of 0.1 mM DPPH solution in each well. The experiment was done in quadruplicate. The plate was shaken and left at room temperature for 30 minutes. After 30 minutes, the absorbance was read at 517 nm wavelength using microplate reader at Central Research and Animal Facilities (CREAM), IIUM Kuantan, Malaysia. DPPH activity was then calculated in percentage using the following formula:

$$\text{Percent of inhibition} = \left[ \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \right] \times 100\%$$

*A blank* = Reading of DPPH solution without crude extract solution

*A sample* = Reading of DPPH solution with crude extract solution

## 2.4 MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (8) Assay

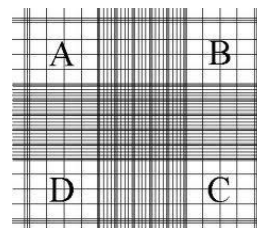
### 2.4.1 Cell Lines and Culture Habitat

MCF-7 cancer cell lines were collected from CREAM. and grown in RPMI 1640 medium supplied with 2mM glutamine, 10mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, 10% FBS (Fetal Bovine Serum) and 50 µg/mL gentamycin in T-25 flasks. The cells were incubated at 37°C in humid atmosphere with 5% CO<sub>2</sub>. The culture was maintained by changing the media twice per week.

### 2.4.2 Cell Counting

After 3 days when the cell reached 70% confluence, the cells were taken out for cell counting. First, the media was taken out from T-25 flask using serological pipette and then media without fetal bovine serum (FBS) was inserted. After removing the media, 1 mL trypsin (Tryp-LE) was inserted to make sure the cell detached. The T-25 flask with trypsin was incubated for 3 minutes before observation under light microscope. The flask was tapped to make sure the cell completely detached from the wall of the flask. Then, 2 mL of growth media without fetal bovine serum was added and all the media with trypsin was transferred into 15mL falcon tube. The cells with media were centrifuged for 5 minutes at 1500 rpm. After 5 minutes, 2.5 mL of supernatant was removed. Then, 500µL of fresh growth media was added into the centrifuge tube. Next, 10µL from the growth media mixture was transferred into microcentrifuge tube and then diluted with 90µL media with 10% FBS. 50µL of the solution was taken for cell

counting on a haemocytometer. The haemocytometer was cleaned using 70% ethanol before being used. The cells were examined under the microscope and counted in the 4 regions using provided formulae:



### Calculation:

Cell count: A: 42 B: 43 C: 46 D: 47 [Total: 178]

The cell densities is:  $\frac{42+43+46+47}{4} \times 10^4 \text{ cells/ml}$

Cell density: 445000 cells/ml

Desired density: 200 000 cell/ml (20 000 cells/ well with 100 µl medium in each well)

Dilution:

$$C_1V_1 = C_2V_2$$

$$(Z)(P) = (200\ 000)(14\text{ml})$$

$$P = (200\ 000 \times 14) / 445000$$

*Z* = acquired cell densities

*P* = total quantity of media with cells needed

### 2.4.3 Cell Seeding

Before the treatment begins, the cells were seeded into 96 well plate. Using provided formula, a total of 14 mL was added into the centrifuge tube. About 200 µL of media with cells from the centrifuge tube was added in each well. The cells were incubated in humidified incubator at 37°C and 5% CO<sub>2</sub> condition for 24 hours.

### 2.4.4 Cells Treatment

Crude extracts were initially diluted with dimethyl sulfoxide (DMSO) before further dilution with growth medium. DMSO used as a solvent because it can dissolve many different compounds. 90 mg of crude extract of each plant sample were diluted with 1mL DMSO in 15 mL centrifuge tube. Then, 40 µL of crude extract diluted with DMSO at different concentrations was transferred into new centrifuge tube. Next, 9.96mL of growth medium with 5% fetal bovine serum was added into the centrifuge tube. The stock solution then was then diluted 6 times in 6 different microcentrifuge tubes using serial dilution to reach desired concentration of between 11.25 to 360 µg/ml. After 24 hours of incubation of cell seeding, the media in the wells were removed. The treatment was added to 6 rows of wells containing cells in quadruplicate. For positive control, 0.06% of H<sub>2</sub>O<sub>2</sub> was used and growth media without any treatment was used as negative control.

### 2.4.5 MTT Assay

After 72 hours of treatment, 20 µl of 5 mg/ml dissolved MTT reagent was added in the wells containing cells for treatment. The plate was incubated again for 3 hours at same condition. After 3 hours, the media was removed and 100 µl of DMSO was added into the treated wells. The absorbance was read using microplate reader at 570nm wavelength. The result was tabulated and the inhibition percentage of the crude extract was determined using formulae below:

$$\text{Cytotoxicity \%} = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100$$

## 3. RESULTS AND DISCUSSION

### 3.1 Extraction of plant samples

In this study, crude of *C. mangga* and *B. rotunda* were extracted using Soxhlet extraction method. A total of 0.126g raw crude extract was obtained from 1g of *C. mangga* dried rhizomes sample. This gives 12.6%

raw crude extract from total weight of the dried sample. Meanwhile, only 0.035g raw crude extract was successfully extracted from 1g of *B. rotunda* dried rhizome's sample. This only gives 3.5% raw crude extract of *B. rotunda* from the total weight of the dried sample. Using absolute ethanol (99.9%) as solvent could possibly have limited the compounds extracted. Extraction of plant sample using 70-80% ethanol produces higher extraction yield, consisting various phenolic compounds [8]. In *B. rotunda*, the most common compounds found in rhizome extracts are pinostrobin, pinocembrin, panduratin C and panduratin A [2]. Meanwhile *C. mangga* extracts, contain flavonoids, saponins, quinone and steroids [3].

**3.2 Antioxidant activity: DPPH assay**

**3.2.1 Antioxidant activity of *C. mangga***

Many analytical antioxidant methods have been developed to evaluate antioxidant properties of plant extracts. One of them is 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH test is based on transfer of electron in solvent that turn the solution into violet. It is soluble in organic solvents. DPPH is a stable free radical that does not easily combine with another molecule [9]. In our experiment, *C. mangga* extract did not reach 50% of DPPH scavenging activity, while the theoretical IC<sub>50</sub> value was higher, 1073 µg/ml. IC<sub>50</sub> value is a percentage value where a treatment can inhibit certain test more than 50% [10].

Based on table 1, DPPH is scavenged starting from lowest concentration of *C. mangga* extract tested. However, the percentage scavenged is not more than 50%, even when the highest concentration of *C. mangga* crude extract used (360 µg/ml). DPPH scavenging activity proportionally increase with the increasing concentration of *C. mangga* extract. Methanolic extract of *C. mangga* rhizome at 40 µg/ml concentration has been reported showing 47.69% inhibition [11]. Based on Muthukumar, et al, 2012, *C. mangga* acetone extract showed 58.63% of DPPH free radicals scavenging at 60 µg/ml concentrations [12]. Methanolic and acetone extracts solvent shows higher percentage of DPPH free radicals scavenging activity compared to using ethanolic solvent [11, 12].

**Table 1:** *C. mangga* ethanolic extract DPPH test

Concentration of <i>C. mangga</i> extract (µg/ml)	Log <sub>10</sub> (sample concentration)	DPPH Scavenging ± SEM (%)	IC <sub>50</sub> Values (µg/ml)
11.25	1.05	6.46±11.70	1073
22.5	1.35	13.68±4.00	
45	1.65	15.3±2.66	
90	1.95	19.9±1.31	
180	2.25	28.4±1.15	
360	2.56	41.5±0.83	

**3.2.2 Antioxidant activity of *B. rotunda***

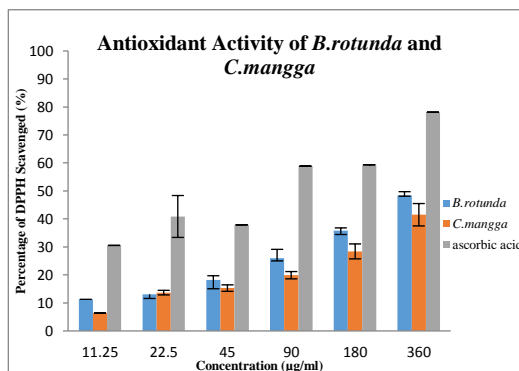
Based on table 2, DPPH is scavenged starting from lowest concentration of *B. rotunda* extract tested. However, the percentage scavenged is not more than 50% even at the highest concentration, 360 ug/ml. The highest percentage of DPPH scavenged is only at 48.38%. DPPH scavenging activity proportionally increased with the increasing concentration of *B. rotunda* ethanolic extract. The IC<sub>50</sub> value of *B. rotunda* is 578 µg/ml which high. Oxygen radical absorbance capacity (ORAC) antioxidant assay of *B. rotunda* extract showed similar antioxidant activity with Trolox at the concentration of 40 µg/ml, the positive control used in a study reported by [2]. Boesenbirgin A, the active compound that could be largely found in the crude extract of *B. rotunda* is attached with aromatic hydroxyl group, which makes this compound as an effective antioxidant. In this experiment, *B. rotunda* was extracted using absolute ethanol, a less polar solvent, while many earlier studies used methanol or acetone, a polar solvent for extraction [2]. Ethanol used as solvent could possibly have extracted less phenolic compounds, a potent antioxidant compound. This lowers the inhibition activity.

**Table 2:** *B. rotunda* ethanolic extract DPPH test.

Concentration of <i>B. Rotunda</i> extract (µg/ml)	Log <sub>10</sub> (sample concentration)	DPPH Scavenging ± SEM (%)	IC <sub>50</sub> Values (µg/ml)
11.25	1.05	11.3± 2.37	578
22.5	1.35	13.1± 1.51	
45	1.65	18.2± 3.14	
90	1.95	26± 0.99	
180	2.25	35.8± 1.36	
360	2.56	48.38± 0.26	

**3.2.3 Comparison of antioxidant activity of *C. mangga* and *B. rotunda***

Antioxidant activity of *B. rotunda* ethanolic extract was found higher than the antioxidant activity of *C. mangga* ethanolic extract (Figure 1). The highest percentage of DPPH inhibition in *B. rotunda* is 48.38% when compared to *C. mangga* at 41.5%. In conclusion, *B. rotunda* ethanolic extract has more antioxidant effect toward DPPH free radicals than *C. mangga* ethanolic extract. however, both of them showed low antioxidant effects as they need higher concentration of crude compounds than previously reported by a group researcher to scavenge DPPH free radical [2].

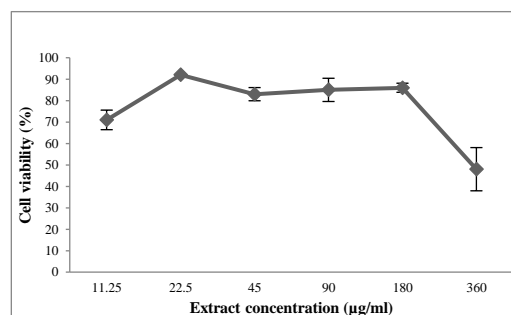


**Figure 1:** DPPH free radical scavenging activity of *B. rotunda*, *C. mangga* and ascorbic acid (positive control)

**3.3 Cytotoxic activity: MTT assay**

**3.3.1 Cytotoxic activity of *C. mangga* ethanolic extracts**

Ethanolic extracts of *C. mangga* at six different concentrations were tested for the *in vitro* anticancer activity against MCF-7 cancer cell lines by using MTT assay method. The cell viability was calculated. Graphical representation of the result is summarised in figure 2 and 3. The data in figure 3 and figure 4 were all taken from a mean of three sets of replicates. The mean readings were analysed into percentage of cell viability to demonstrate cytotoxicity of the rhizome ethanolic extracts of both *C. mangga* and *B. rotunda*.



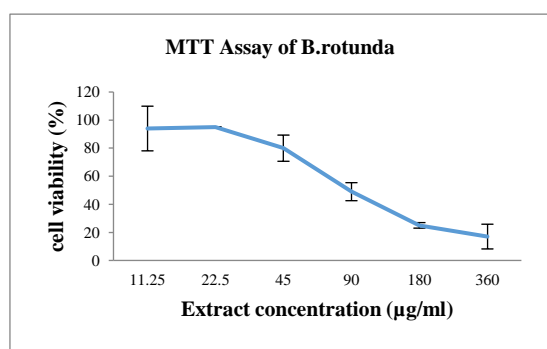
**Figure 2:** MCF-7 cancer cells viability after treated with *C. mangga* ethanolic extracts

The result revealed that *C. mangga* ethanolic extract at the concentration of 360 µg/ml has the lowest cell viability of 48%. However, the overall graph pattern fluctuates between the concentration of 11.25 µg/ml and 180 µg/ml at 72% to 86%, showing no significant decrease of cell viability. Only at the highest concentration of 360 µg/ml the cell growth inhibited to below 50%. Thus, the result shows that the cell viability decreases at the concentration of extract of 360 µg/ml. The result could be influenced by the solvent used in extraction of *C. mangga* sample. The higher the concentration of crude needed for treatment indicates the poor cytotoxic effect of this plant extract.

MTT measures cell respiration and the amount of formazan produced by the cell and it is proportional to the number of living cells present in culture. For the treatment, the proportional decrease in the number of cells with increasing treatment concentration demonstrates higher or increasing cytotoxicity of the extract [13]. A group researcher reported the positive cytotoxicity effect of *C. mangga* acetone extract against MCF-7 cell [12]. Meanwhile in other study also reported positive cytotoxicity effect of methanolic extract of *C. mangga* against MCF-7 [14]. Thus, different extraction solvent could give different amount of compound present thus, the biological activities could be different.

### 3.3.2 Cytotoxic activity of *B. rotunda* ethanolic extracts

Ethanolic extracts of *B. rotunda* at six difference concentrations were tested for the *in-vitro* anticancer activity against MCF-7 cancer cell lines using MTT assay. The cell growth inhibitions were calculated. Graphical representation of the result is summarised in figure 3.



**Figure 3:** MCF-7 cancer cells viability after treated with *B. rotunda* ethanolic extracts.

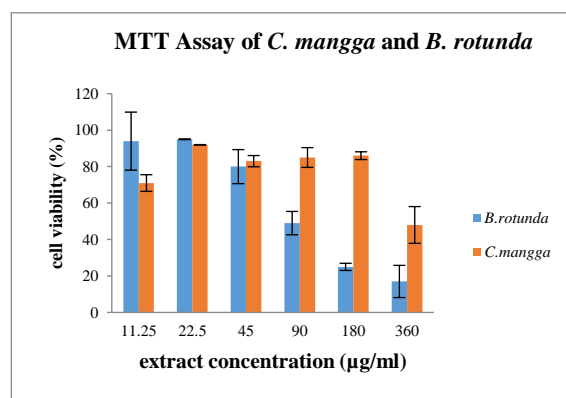
Meanwhile for *B. rotunda*, the graph showed clear pattern of cell viability. The number of cells inhibited proportional to the increasing concentration of *B. rotunda* ethanolic extracts. At the concentration of 360 µg/ml cancer cell viability at the percentage of 13.53%, which portrays ethanolic extract *B. rotunda* as promising cytotoxic agent. According to a study, *B. rotunda* ethanolic extract showed positive antiproliferative effect against five cancer cell lines including MCF-7 with IC<sub>50</sub> ranging from 51 to 71 µg/ml [15]. They also found that the rhizome extract showed cytotoxicity when compared to the leaves extract. This proves that the active compounds with cytotoxic properties are found in the rhizomes.

MTT assay are used to observe the cell viability after treatments with plant extracts. MTT assay is part of tetrazolium salts that are widely used in histochemistry and biotechnology fields. Metabolism of cells will produce NADPH dependent oxidoreductases and dehydrogenases. This will lead to production of formazan in MTT solution and will turn the solution into blue or purple [16].

### 3.3.3 Comparison of cytotoxic activity of *C. mangga* and *B. rotunda*

In comparison, *B. rotunda* extracts shows a strong inhibition of MCF-7 cancer cells than *C. mangga* extracts (Figure 4). The minimum cell growth or viability in *B. rotunda* extract is 13.53% while on *C. mangga* extract is 48%. This could be due to the compounds present in the extracts, especially in *B. rotunda* which has many specific compounds such as boesenbigin A, pinostrobin and panduratin. These compounds have different effects of cytotoxic activity [17]. *C. mangga* have a higher IC<sub>50</sub> value which is 207 µg/ml than compared to *B. rotunda* extract which

is 31.96 µg/ml. This means *B. rotunda* has higher cytotoxic effect toward MCF-7 cells than *C. mangga*.



**Figure 4.:** Graph of cell viability by two extract treatments; *B. rotunda* and *C. mangga*

## 4. CONCLUSION

This study shows that ethanolic extracts of *C. mangga* and *B. rotunda* have potent antioxidant activity. However, *C. mangga* and *B. rotunda* crude extracts shows distinctive different on cytotoxic effect towards MCF-7 cancer cells. *B. rotunda* crude extracts showed higher cytotoxic and antioxidant effect than *C. mangga* extracts.

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