



## Selective Cytotoxic Activity of Methyl-3,4,5-trihydroxybenzoate Isolated from Kernel of Bambang (*Mangifera pajang*)

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Bambang (*Mangifera pajang*) has been shown to exhibit anticancer activity. One of the major bioactive compounds present in the methanol extract of kernel of *M. pajang* is methyl 3,4,5-trihydroxybenzoate (methyl gallate). The present study was conducted to evaluate the cytotoxic activity of this compound against selected cancer cell lines such as hormone dependent breast cancer (MCF-7), non-hormone dependent breast cancer (MDA-MB-231), prostate cancer (PC-3), pancreatic cancer cell (CP-2) and colon cancer (HCT-116) cell lines. Methyl gallate was isolated from methanol extract by using column chromatography and the compound was further confirmed by using NMR, GC-MS analysis and comparison of spectral data of the isolated data with published report. The cytotoxicity of the compound was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay at concentrations ranging from 0 to 100  $\mu$ M. The results showed that the compound only induced cytotoxicity in MCF-7 and PC-3 cell lines with  $IC_{50}$  values of  $54.7 \pm 4.73$  and  $97.6 \pm 4.04$   $\mu$ M, respectively. Furthermore, Capan 2, MDA-MB-231 and HCT-116 showed no inhibition towards the cell proliferation after the treatment with compound ( $IC_{50}$  values more than 100  $\mu$ M). Thus, the compound isolated from kernel of *M. pajang* exhibited selective cytotoxic activity against selected cancer cell lines.

**Keywords:** Methyl gallate, *Mangifera pajang*, Cytotoxicity, MTT assay, Cancer cells.

### INTRODUCTION

Cancer is a general term that used to describe a group of diseases that can affect any parts of the body. It has been found to be the fourth leading cause of death in Malaysia. Cancer can be developed by many factors such as radiation, tobacco and exposure to chemicals. Not only that some internal factors may be the cause to cancer to be worsen such as hormone imbalance, inherited mutation and immune system [1]. To date, in order to combat cancer there are several significant treatments that are available by surgery, chemotherapy and radiotherapy [2]. However, drugs that been used in chemotherapy (such as doxorubicin and cisplatin) were found to be high in toxicity and can induce side effect both on tumorous and normal cell as well [3]. Thus, it is clear that the investigation of plant

derived anticancer compound giving the opportunity for the development of anticancer drugs from the natural resources, which have long being used traditionally to treat many diseases.

*Mangifera pajang*, also known as Bambang is a species of mango group which widely can be found in Borneo Island of Malaysia (Sabah-Sarawak), Indonesia (Kalimantan) and Brunei [4]. The natives of Island have practically consuming fruit of this plant as their staple food by making the flesh as pickle. Not only that the grated kernel that have been fermented for several days are added and eaten with rice [5]. A review by Abu Bakar and Fry [6] reported that the *M. pajang* contained diverse phytochemicals and display various promising health benefits. In previous study, it has been reported that kernel extract of this plant consisting high level of phenolic phytochemicals [7] such as caffeic acid, chlorogenic acid, *p*-coumaric acid,

gallic acid and sinapic acid as well as flavonoids groups such as diosmin, hesperidin, naringin and rutin [8]. A related study also reported that only the kernel and peel extracts exhibit cytotoxic effects on proliferation of liver and ovarian cancer cell lines with an  $IC_{50}$  value ranging from 35-92  $\mu\text{g/mL}$  [8]. Different fruit parts of *M. pajang* such as peel, pulp and kernel displayed antioxidant [7], antibacterial and anticancer activities [6,7].

There are many studies on the plant extract and isolated compounds been tested *in vitro* and *in vivo* for the cytotoxicity purposes by using different human cell lines including liver colon, prostate and breast cancer cell lines. The evaluation of cytotoxicity of plant extract and isolated compounds are crucial as they bring effect to the drug discovery [9]. Literature search have revealed numbers of compounds previously have been isolated from this plant species but not with the anticancer activity from *M. pajang*. Herein, this study part was aimed in anticancer activity of methanol and ethyl acetate extract. Moreover, lead compound belong to phenolic acid was isolated from the active extract and its anticancer properties towards proliferation of selected cancer cell lines was determined.

## EXPERIMENTAL

**Extraction and isolation:** Plant materials were collected from Penampang, Sabah, Malaysia and identification was done by Dr. Mohd Fadzelly Abu Bakar from Universiti Tun Hussein Onn Malaysia (UTHM). Plant kernel of *M. pajang* were separated from its flesh and peel before dried and ground into powder (800g). Isolation of bioactive compound was conducted using method of Ahmad *et al.* [4].

**Cell culture:** All cells used were obtained from ATCC and maintained in RPMI 1640. Meanwhile, Capan 2 carcinoma cell was maintained in McCoy's 5a supplemented with 10 % fetal bovine serum, 1 % penicillin-streptomycin at 37 °C in 5 %  $\text{CO}_2$  incubated in a humidified incubator [10]. The cultures were closely monitored under inverted microscope until the cell reached 80 % confluence. Adherent cancer cells approaching 80 % confluency were harvested by washing with phosphate buffer saline and followed by trypsin to detach the cell from the culture flask. The harvested cells were then collected in a fresh medium and sub-cultured at densities of  $1.0\text{--}5.0 \times 10^5$  cells/mL into 25  $\text{cm}^2$  flask as being adopted by Stanslas [11].

**Cytotoxicity activity using MTT assay:** The log phase cells with viability of 80 % were trypsinized to detach the cells from the culture flask. Cells were then collected in a fresh medium and the number of the cells were determined. About 2000 cells in 180  $\mu\text{L}$  of medium were seeded into each well of 96 well plate and incubated for 24 h before the drug was introduced. The stock solution of  $10^5 \mu\text{g mL}^{-1}$  were prepared by using dimethyl sulphoxide of concentration ranging from  $1000 \mu\text{g mL}^{-1}$  to  $1 \mu\text{g mL}^{-1}$ . Working solution were prepared by 10 times dilution of stock and 20  $\mu\text{L}$  of these concentrations was added into each well yield a final concentration ranging from ( $100 \mu\text{g mL}^{-1}$  to  $0.1 \mu\text{g mL}^{-1}$ ). The final mixture used for treating the cell contained not more than 0.1% of the solvent, the same as for the control wells. The plates were then incubated at 5 %  $\text{CO}_2$ , 37 °C under high humidity for 4 days and the viability of cells in the plates were determined. 50  $\mu\text{L}$  of MTT solution (5 mg

$\text{mL}^{-1}$ ) were added into each well and incubated for another 4 h. The medium was aspirated and crystal formazan precipitate was dissolved in DMSO. The plate was then analyzed using SOFTProMax spectrophotometer. All experiments were performed in triplicates. The  $IC_{50}$  was generated from graph of cell viability against concentrations of compound.

## RESULTS AND DISCUSSION

**Isolation of compound:** By referring the isolation method of Ahmad *et al.* [4] with slight modification, pure compound of methyl gallate (20.3 mg) was isolated from methanolic extract of kernel of *M. pajang* by using column chromatography (unpublished report). The spectroscopic data of isolated compound was analyzed by using nuclear magnetic resonance (NMR), gas-chromatography mass spectrometer (GC-MS) analysis and direct comparison of spectral data of isolated compound with the published report of Daud *et al.* [14]. The isolated compound was identified to be methyl-3,4,5-trihydroxybenzoate (methyl gallate). This compound has been widely reported to be the major components in the *Mangifera* species and displayed the best cytotoxicity activity due to carbonyl and three hydroxyl groups which attached to benzene ring [4]. Phenolic compounds (caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid and sinapic acid) and flavonoids (diosmin, hesperidin, naringin and sinapic acid) have also been reported to be the major groups of phytochemicals in *M. pajang* kernel [12].

The isolation of compound from the present study has provided an additional support for the previous study of Ahmad *et al.* [4], where isolation of identical compound from the same active extract were reported. This compound also been reported to occur in ethanolic extract of *Galla rhois* [13], *Labisa pumila* Benth. [14] and known to have antimicrobial properties [4] and strong antioxidant properties [15].

**Cytotoxic potential:** The cell viability was analyzed by using MTT assay originally described by Mosmann [16], has been used to define quantitative colorimetric assay for cell survival and proliferation based on reduction of tetrazolium salt to purple insoluble formazan. These cell lines were tested with crude extract at the highest concentration of 100  $\mu\text{g/mL}$ . Based on the dose response curve, polar solvents (ethyl acetate and methanol extracts) shown good cytotoxicity on MCF-7, HCT116, PC3 and Capan 2 (Table-1). This may be due to the presence of cytotoxic methyl gallate constituents. Meanwhile, hexane and chloroform extracts were considered no cytotoxicity towards the cancerous cell lines, which exhibited  $IC_{50}$  values greater than 100  $\mu\text{g/mL}$ . In comparison with the present study, one of the active extracts (ethyl acetate: 21  $\mu\text{g/mL}$ ) was much lower compared to  $IC_{50}$  value of ethanolic extract from the previous study of Abu Bakar *et al.* [8] when treated towards the same cancer cell line (MCF-7). Moreover, different solvent used in the extraction method may influence the types of compounds that may present in the plant extract. In agreement with this study, the same trend of cytotoxicity was also reported by Ahmad *et al.* [4] which the cytotoxicity of both extracts were less than 10  $\mu\text{g/mL}$  against MCF-7, HeLa and HT29 cell lines.

From the isolated compound, only MCF-7 and PC-3 were inhibited by methyl gallate at the highest concentration tested (100  $\mu\text{M}$ ). The results (Table-2) obtained indicated that the

TABLE-1  
IC<sub>50</sub> OF THE CRUDE EXTRACTS AND ISOLATED COMPOUND FROM EXTRACTS OF  
KERNEL OF *M. pajang* AFTER 4 DAYS ON SEVERAL CELL LINES

Extract	IC <sub>50</sub> (µg/mL)				
	MCF-7	MDA-MB231	PC3	Capan 2	HCT116
Hexane	> 100	NA	> 100	> 100	> 100
Chloroform	> 100	NA	> 100	> 100	> 100
Ethyl acetate	31.67 ± 3.06	NA	31.67 ± 3.51	17.67 ± 4.73	41.33 ± 2.08
Methanol	34.67 ± 2.31	NA	30.0 ± 2.0	54.33 ± 9.01	34.44 ± 2.08

treatment of methyl gallate on other cancerous cells (MDA-MB231, Capan 2 and HCT116) have little or no toxicity by exhibiting IC<sub>50</sub> values of greater than 100 µM. Similarly, MCF10A cells also demonstrated IC<sub>50</sub> value of > 100 µM.

TABLE-2  
CYTOTOXICITY ACTIVITIES OF METHYL GALLATE  
TOWARDS SELECTED CANCER CELL LINES

Cell lines	IC <sub>50</sub> (µM)
MCF-7	54.7 ± 4.73
MDA-MB231	> 100
PC3	97.6 ± 4.04
Capan 2	> 100
HCT116	> 100

As shown in Table-2, methyl gallate exhibited the lowest IC<sub>50</sub> value in MCF-7 (54.7 ± 4.73 µM) indicating that it marked cytotoxic effects on hormone dependent breast cancer cell. The less cytotoxic effects of methyl gallate was exhibited in prostate cancer cell with IC<sub>50</sub> value 97.6 ± 4.04 µM. This finding is consistent with the observation of Ahmad *et al.* [4] which reported that there are inhibition of cell growth of MCF-7 and colon cancer cell at the concentration of lower than 20 µg/mL by similar compound. Methyl gallate have also been isolated from the seed kernel of *Mangifera indica* Linn. cultivar Chok-Annan as the main phenolic acid and flavonoid and exhibit potent antioxidant properties [17]. Besides, it was reported that methyl gallate is an excellent antioxidant and inhibit lipid oxidation [18]. Not only that methyl gallate also reported to exhibit good anti-bacterial properties by inhibiting the growth of intestinal bacterial such as *Clostridium perfringens*, *Escherichia coli* and *Salmonella typhimurium* [19].

In addition to anticancer activity of methyl gallate, further investigation on the antioxidant of this bioactive compound also being conducted in the previous study, where it showed the strong scavenging activity with an IC<sub>50</sub> value of 2.8 µg/mL [20]. The activity of isolated compound probably has a strong structure-activity relationship as suggested by Fiuza *et al.* [21]. In conjunction with the recent study, this assay is important to define the basal cytotoxicity of this compound towards the cancerous cell activity such as intrinsic ability of this compound to cause cell death as a result of damage to several cellular functions. The assay was evaluated based on the intensity of purple formazan crystal, which is a reduced-form of water soluble tetrazolium salt (MTT) [22-25] measured directly to proportional to the number of viable cells in the culture [26-28]. This preliminary data also important to define the concentration range for more details *in vitro* study to provide information on the molecular mechanisms underlying that may contributed to anticancer role of methyl gallate [29].

The community of Kadazan-Dusun people in Sabah, Malaysia has developed variety of traditional cuisine using fresh and preserved fruits of Bambang (*M. pajang*). Several studies on phytochemicals and pharmacological properties of this fruit had affirmed that regular consumption of Bambang fruits among Kadazan-Dusun people would give various health benefits. Functional food products from Bambang fruits also being developed in Sabah, Malaysia. Not only that, present study could serve as preliminary data towards developing of pharmaceutical applications of the by-products (kernel), which are abundant in supply following consumption of edible parts of fruits.

## Conclusion

In conclusion, screening of cytotoxicity of the crude extracts revealed that the methanolic and ethyl acetate extract exhibited significant anticancer activity against MCF-7, HCT116- Capan 2 and PC3 cell lines. This can also be the evidence for the presence of effective anticancer agent, which have been isolated and identified as methyl gallate might be contributing to the anticancer property. Despite the selective cytotoxicity displayed by the active compound, further research is needed to be conducted as methyl gallate may be useful in breast cancer treatment and prevention.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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