# Korespondensi Penulis dengan
# European Review for Medical and Pharmacological Sciences

**Article History**

| | |
|---|---|
| Submission | : 23 Juni 2022 |
| Hasil Review | : 24 Agustus 2022 |
| Pengiriman Revisi | : 31 Agustus 2022 |
| Accepted | : 02 September 2022 |
| Galley Proof-1 | : 20 September 2022 |
| Galley Proof-2 | : 07 Oktober 2022 |
| Published | : 15 Maret 2023 |

# Article #27067 submitted

**Publishing Manager** <info@publishingmanager.org>
Reply-To: "info@publishingmanager.org" <info@publishingmanager.org>
To: hendrik-s-b@fkg.unair.ac.id

Thu, Jun 23, 2022 at 7:49 AM

Dear Doctor,

Thank you for submitting your article "Palmitic acid of Musa Paradisiaca induce apoptosis through caspase-3 in human oral squamous cell carcinoma" to European Review for Medical and Pharmacological Sciences.

We are in the process of evaluating your manuscript. We evaluate all manuscript submissions as expeditiously as possible and appreciate your patience throughout the peer-review process.

Best regards,
Journal Editorial Team

**Abstract**

**Objective:** Despite apoptosis processes being conserved, cancer cells have developed mechanisms to inhibit apoptosis by altering anti apoptotic molecules or inactivating pro-apoptotic. The aim of this study was to determine the palmitic acid of *Musa paradisiaca var. sapientum (L) Kunz* (MP) stem extracts against human oral squamous cell carcinoma (hOSCC) through caspase-3.

**Materials and Methods:** Ethanol and ethyl acetate extracts of MP stem were analyzed by gas chromatography-mass spectrometry (GC-MS). Computerized models of chemically active compounds were used to predict anticancer activity. Cytotoxicity was evaluated in *Artemia salina Leach* and hOSCC (OM-1) culture at concentrations 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL respectively. The expression level of caspase-3 on hOSCC was measured by Elisa.

**Results:** We found seven chemically active compounds in the ethanol extract and 15 compounds in the ethyl acetate extract of MP stem. The major component was hexadecanoic acid of palmitic acid derivates, and this was predicted to have anticancer activities as apoptosis through caspase-3 stimulants. However, cytotoxicity effects against hOSCC culture were assessed by values of the 50% inhibitory concentration ($IC_{50}$) of 15.00 µg/mL for the ethanol extract, and an $IC_{50}$ of 10.61 µg/mL for the ethyl acetate. There was increasing significantly of caspase-3 level on treatment groups compared than control.

**Conclusions:** Hexadecanoic acid of MP stem extracts have anticancer activity by inhibiting cell growth of hOSCC culture through caspase-3 stimulants.

**Keywords:** oral squamous cell carcinoma; anticancer; palmitic acid; apoptosis; caspase-3

## Introduction

Oral cancer is a common cancer in both developing and developed countries when people undergo lifestyle changes[1]. One of the prominent types of oral cancer is oral squamous cell carcinoma (OSCC), which has reached a high number of cases in Southeast Asia with a prevalence of more than 90% among oral cancers[2]. OSCC occurs place in any region of the oral cavity, pharyngeal region and salivary glands[3]. It was traditionally associated with certain risk factors, such as heavy smoking, consumption of betel nut and alcohol[4], an impacted wisdom tooth, poor dental fillings, ill-fitting denture prostheses[5], unhealthy diet and nutrition[6], and the human papillomavirus (HPV) infection[7].

Irritating chemicals and free radicals as stress signals received by intercellular molecules mediate mitochondrial outer membrane permeabilization and released pro-apoptotic molecules into the cytoplasm[8]. The B-cell lymphoma (Bcl-2) family and inhibitors of apoptotic proteins that serve as apoptotic switches, control the permeabilization of mitochondrial membranes[9]. Some suggested that these two pathways may work in synchronization to initiate apoptosis. Despite apoptosis processes being conserved, cancer cells have developed mechanisms to inhibit apoptosis by altering anti apoptotic molecules or inactivating pro-apoptotic cell death components through decreasing of caspase-3. Cancer cells had adopted several mechanisms to evade apoptosis[10]. These mechanisms can be windows into the development new drugs as cancer therapy.

Chemotherapy and radiotherapy are first-line treatment regimens to manage OSCC. However, these conventional therapies have several severe side effects due to their non-specific actions against highly proliferating normal cells. It is been well known that nausea, vomiting, hair loss, Candida infections, and epithelial-ulceration are common manifestations of adverse effects derived from the use of anticancer drugs such as cisplatin, carboplatin, 5-fluorouracil[11], docetaxel, paclitaxel, and methotrexate[12], and radiation-induced xerostomia[13]. Thus, many

studies have been conducted to develop non-toxic herbal medicines as alternative to chemical drugs, with the hope of reducing severe side effects in cancer patients.

Plants contain a broad array of phytochemicals with various functional and non-functional activities, and plant extracts might be subjected to particular prescreening prior to detailed investigations. One of methods to screen the active chemical compounds in medicinal plants is through gas chromatography-mass spectrometry (GC-MS)[14]. GC-MS is a key technique to detect and separate volatile compounds, including secondary metabolites in both plant and non-plant species[15,16]. In addition to the intense laboratory-based screening and analytical processes, the emergence of computational screening that utilizes artificial intelligence systems has expedited the development of new candidates through drug discovery. Database applications such as *Prediction of Activity Spectra for Substances* (PASS SERVER), a web-based application is being used to evaluate the biological potentials of organic molecules that can be developed as medicines[17,18]. Prediction results of PASS SERVER are presented as values of Pa (probable activity) and Pi (probable inactivity), with the Pa>Pi ratio indicating a good probability of predicted activity[19]. The robustness of this technique made the screening of new anticancer drugs more feasible, and additional analytical assistance from *in silico* prediction of cell line cytotoxicity (CLC-Pred tools) adds value in predicting the toxicity to cancer cell cultures[20]. Chemically active compounds with potential anticancer activity that are sorted using GC-MS and computational (*in silico*) modeling should be tested for their biological and toxicological activities. The brine shrimp lethality test (BSLT), a bioassay that screens the cytotoxicity of a specific compound or extract, uses shrimp larvae of *Artemia salina Leach* to determine the 50% lethal concentration ($LC_{50}$) through probit analysis[21-23]. A 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) in vitro cell proliferation assay is one of the most extensively used methods for assessing preliminary anticancer activities of synthetic derivatives, natural medicines and natural product extracts. This colorimetric-based test is highly reliable and can be used on a wide range of cell lines. This assay indicates whole-cell cytotoxicity; however, additional experiments were required to define particular molecular target[24,25].

Banana trees are tropical plants that are often used as herbal medicines. The banana tree can be used as an alternative treatment of wounds, fevers, insect bites, digestive disorders, and epilepsy[26]. The sap of the Ambonese banana (*Musa paradisiaca var. sapientum (L) Kunz*) (MP) stem has long been used to accelerate wound healing and relieve tooth pain in the village community of Trunyan, Bali, Indonesia[27]. There are significant compounds in banana plants, such as saponins, flavonoids, anthraquinones, and tannins contained in both the fruit and sap, as well as lectin[28]. Sap aspirated from the stem of the MP was shown to have many therapeutic activities, including wound healing[29]. Since high levels of quercetin and lectin are found in banana sap, it was shown to suppress cancer cell proliferation, increase macrophage activity, inhibit matrix metallopreinases (MMP)-2 and -9 activities[30,31], increase T cell proliferation[28], increase fibroblast activity and promote angiogenesis[29].

In the present study, new chemically active compounds derived from the extract of MP stem were screened using GC-MS methods, *in silico* prediction activity, the in vitro BSLT and MTT assay, and also apoptotic activity of caspase-3 as potential candidates for anticancer drug.

## Materials and Methods

### *Preparation of MP Stem Extract*

Stems of MP were cleaned and chopped to a size of 0.5-1 cm. Then, the stems were dried in an oven at a temperature of 50 °C for 3 h. The dried stems were blended to become a powder form. Maceration was conducted with 96% ethanol or acetic acid as solvent for 7 days for each solvent. First, 1 kg powder of MP was mixed with 2 L of 96% ethanol and 1 kg powder was mixed with 2 L of 96% ethanol and 20 mL of glacial acetic acid. All process required 7 days

and were conducted twice for each solvent. The product was filtered through a no. 41 Whatman membrane (Merck, USA). The extract and solvent were separated by a rotary evaporator (Heidolp) at a temperature 50 °C and a speed of 200 rpm for 2 h until a condensed extract was obtained. This was placed in a covered container to avoid light[32].

***Preparation of Stock Solutions***

This study was carried out with 10-100 µg/mL as working concentrations. First, a stock solution was made by dissolving 1000 mg of MP stem extract in 1 L of sterile distilled water (1000 mg/L=1000 ppm), and then 1 mL of the stock solution was mixed with 9 mL of sterile distilled water (DIW) to make a concentration of 100 ppm (100 µg/mL). We then mixed 0.9 mL of the stock solution with 9.1 mL of sterile DIW to make 90 ppm (90 µg/mL). The same preparation methods were used to obtain concentrations of 80, 70, 60, 50, 40, 30, 20 and 10 µg/mL[33].

***Analysis of Chemically Active Compounds by GC-MS***

Stem extract of MP was analyzed by GC-MS (Shimadzu QP 5000, Japan). GC-MS spectroscopy was operated using a capillary column with a height of 25 cm, a diameter of 0.25 mm, and a thickness of 0.25 µm. A sample (1 µL) was injected into the mobile phase (helium gas, at a pressure of 12kPa), and it carried the sample mixture to the stationary phase (CP-Sil 5 CB). The capillary column was in an oven that was programmed to gradually increase the temperature (70 to 270 °C), at a rate of 10 °C/min. Total separation of the mixture components was 30 mL/min, and thus a chromatogram was produced representing an m/z ratio of 1:50[14].

***Predictions of Chemically Active Compounds of MP Stem Extract on PASS the SERVER***

The National Centre for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/) was used to obtain chemical structures of selected phytoconstituents of MP stem extracts. Selected structures were downloaded as sdf files for prediction in PASS SERVER software (vers. 2.0; http://www.way2drug.com/PASSonline/), and a compound's predicted activity spectrum was used to calculate the probable activity (Pa) and probable inactivity (Pi). The prediction of this spectrum by PASS SERVER was based on a structure-activity relationship (SAR) analysis of a training set containing over 205,000 compounds with over 3750 different biological activities. Pa and Pi values range 0.000-1.000 because they are probabilities. Generally, $Pa+Pi \neq 1$ since these probabilities were calculated independently[17].

***Preparation of Shrimp Larvae Culture (A. salina Leach)***

Artificial seawater was prepared. Briefly, shrimp larval eggs were dropped into an aquarium which contained NaCl (15 g) in 1 L of DIW at a neutral pH (8-9). The container placed in the room with light, and the temperature was maintained at around 25-31 °C. After 48 h the eggs hatched and the larvae swam to the lighted area[22]; therefore, the larvae could be separated from their shells and then healthy larvae were cultivated and used to test the extracts of MP.

***Toxicity Test by the BSLT***

A brine shrimp (*A. salina Leach*) lethality assay is commonly used to check cytotoxic effects of bioactive chemicals. Shrimp eggs (0.25 g) were weighed on an analytical balance, and then grown in 300 mL of artificial seawater for 1-3 days. After 1-3 days, the eggs hatched, and became larvae (nauplii), and we selected healthy larvae for subsequent experiments. Concentrations of 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL of extracts of MP stems in artificial sea-water were prepared and placed in test tubes containing 10 nauplii, Nauplii were

evaluated, and dead ones were counted after 24 h. A dead larva indicated by a shrimp larva which did not show activity for a few seconds of observation[22].

***Pre-Culture of hOSCC Cells in T75 Flask***

*Human oral squamous cell carcinoma* (OM-1) cells stored in the vial preserved in a deep freezer (at -80 °C). Cells were thawed in sterile DIW at 37 ºC and moved to Falcon tubes with 5 mL of cell medium. The Falcon tubes were centrifuged at 500 rpm for 5 min. Cell medium consisted of high-glucose Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) and fetal bovine serum (FBS; Sigma-Aldrich) in a 90:10 ratio. The supernatant was removed and 5 mL of new medium was carefully added to each Falcon tube. The pellet (containing hOSCC cells) was gently resuspended in the medium and seeded into a flask. The flask was incubated for 2-3 days at 37 ºC and 5% $CO_2$ until a confluent monolayer of cells (80% density) was detected with an inverted microscope at 100x magnification (Nikon, Japan). Cells was detached using TrypLE™ Select Enzyme (Gibco, USA), harvested and counted with a cell counter (Scepter; Sigma-Aldrich), and the cells density was $1.3x10^6$/mL.

***Anticancer Activity Screening of MP Stem Extract against hOSCC Cells by An MTT Assay***

The microplate containing hOSCC cells was observed under an inverted microscope at a 100x magnification to ensure that cells were confluent. Each group consisted of three replicate wells (triplo). Treatment groups contained the MP stem ethanol extract or ethyl acetate extract at 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL. Two control groups were control medium that contained culture medium without cells and a control cell group that only contained cells and culture medium. Then, 25 µL of an extract was added to each well and incubated for 24 h at 37 ºC, and 5% $CO_2$. To each well was added 10 µL MTT (M2003; Sigma-Aldrich), and then the mixture was covered with aluminum foil and incubated for 4 h at 37 ºC. Lastly, 50 µL DMSO was added to each well and shaken vigorously, and then the absorbance level was measured by an enzyme-linked immunosorbent assay (ELISA) reader at a wavelength of 620 nm to obtain the $IC_{50}$ value[34].

***Measuring the Caspase-3 Level on Hoscc Cells Treated MP Stem Extract by ELISA***

The apoptotic activity of caspase-3 was measured with ELISA on hOSCC cells culture. The hOSCC cells were initially grown in 6-well plate with a seeding density of $4.10^5$ cells per well (2 ml volume). When the cells reached 85-90% confluence, cells were treated with 6 different concentrations of MP stem ethanol extract (0, 22.5, 45, 90, 360 ng/ml) and incubated for 0-, 2-, 24-, and 48-hours in 37 ºC, 5% $CO_2$. The complete DMEM growth medium without penicillin/streptomycin was used during the treatment. Every time points of treatment, a 1 ml of supernatant was collected for ELISA analysis. The sandwich ELISA kit (Bioenzy, Cat. BZ-08124084-EB) was used for a quantitative detection of Human Caspase-3. The collected supernatant was preliminary centrifuged to remove the debris and later transferred to 96-wells ELISA plate. The sample preparation was done by following the assay procedures described in the kit. Each sample was done in triplicate. As controls, untreated cells and serum-free medium were applied. Absorbance was determined at 450 nm using a microplate spectrophotometer (BioTek, Epoch, USA).

***Statistical Analysis***

Data of larvae mortality were tabulated and assessed to obtain the $LC_{50}$ using a probit analysis[21]. However, the absorbance of the hOSCC culture was measured to obtain the $IC_{50}$ and caspase-3 level. All data were analyzed by GraphPad Prism 8th software. The concentration relationship between the ethanol and ethyl acetate extracts was assessed using a linear regression at a 95% confidence interval.

## Results

### *Chemically Active Compounds of MP Stem Extract by GC-MS*

The components of the extracts were identified by comparing peak values of mass spectral fragmentation patterns to those of reference compound fragmentation patterns from the *Wiley Registry of Mass Spectral Data*. Several chemically bioactive compounds were identified in the ethanol extract of MP stems (Figure 1). The main components were 11.47% of 9, 12 octadecadienoic acid, 5.40% of hexadecanoic acid, methyl ester and pentadecanoic acid, 14-methyl which is palmitic acid (a group of saturated fatty acids). Meanwhile, other fatty acid groups with less than 5% concentrations included n-hexadecanoic acid, methyl stearate, heptadecanoic acid, 16-methyl, methyl ester, heptadecanoic acid, 14-methyl, methyl ester, and benzo (h) quinoline, 2, 4-dimethyl (Table 1).

There were 15 peak values found by GC-MS in the ethyl acetate extract, but the concentrations were less than 5% (Figure 2). The main constituents were from the fatty acid family, with 4.62% oleic acid and octadec-9-enoic acid and, 2.08% 9.12-octadecadienoic acid (z, z). Using ethyl acetate as a solvent increased the extraction of chemically bioactive compounds from MP stem of not only palmitic acid, but also 1.21% of ethanol, 2-bromo, and other compounds with concentration of < 1%, such as 0.81% of 3'-chlorooxanilic acid N'(3-ethoxy-4-hydroxybenzylidene) hydrazide, 0.44% of 2-propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-3, 0.28% of cis-2,4,5-trimethoxy-.beta.-methyl-.beta.-nitrostyrene, 0.25% of 4-fluorobenzoic acid, 2-phenylethyl ester and 0.20% cyclopropaneoctanal, 2-octyl-(Table 2).

### *Prediction of Biological Activities of Chemically Bioactive Compounds from MP Stem Extracts Using the Online PASS SERVER*

Some potentially bioactive compounds could be estimated through predictions using the online PASS SERVER. Based on results of chemically bioactive compound screening found using the GC-MS analysis, in general, compounds were members of the fatty acid group, such as *palmitic acid, linoleic acid, oleic acid,* and *stearic acid*. Chemically bioactive compounds with predicted potential were those that had a percentage of similarity to comparison database compounds of ethanol extract (Table 1) and ethyl acetate extract (Table 2) of MP stem that were greater than 90%. A compound's potential was predicted using a canonical smile obtained from the *PubChem online service* (Table 3).

Predictions were based on results of online computing according to the PASS SERVER, and four fatty acid compounds had potential that could be used as a starting point of this study for anticancer agents. These compounds were palmitic acid, linoleic acid, enolic acid, and stearate. They were identified by examining Pa value in the activity of superoxide dismutase (SOD) inhibitors, apoptosis agonists, and caspase-3/caspase-8 stimulants. The data revealed that the bioactive compounds of hexadecanoic acid (palmitic acid) and pentadecanoic acid, 14-methyl, methyl ester had the highest Pa values against the activity of SOD inhibitors at Pa=0.914. The bioactive compounds with the highest apoptosis agonist activity were 9.12-octadecadienoic acid (z,z), methyl ester and cis-13-octadecadienoic acid, methyl ester (linoleic acid) with Pa=0.545. Furthermore, the bioactive compound with the highest activity as a caspase-3/caspase-8 stimulant was linoleic acid (Pa=0.617) (Table 3).

The predicted result of bioactive compounds obtained Pa>0.7, indicating that the test compound's activity was the same as the drug's, and there were bioactive compounds with values of 0.5<Pa<0.7, indicating that the test compounds had lower biological activities than the drug. Table 4, shows predictions of hexadecanoic acid (palmitic acid) compounds with toxic activity against cancer cell cultures (lung carcinoma, metastatic melanoma, melanoma,

and stage 3 non-small cell lung cancer cells). In our results, predictions of toxic compounds from MP stem extract using CLC-Pred tools showed Pa>0.5.

***BSLT Results of MP Stem Extracts***

Brine shrimp lethality test (BSLT) is an acute toxicity test that applies a probit analysis to determine toxic concentration within 24 h. At each concentration, the death of shrimp larvae was measured, and a concentration-response relationship was determined using a regression analysis. The following equation was used to calculate the toxicity of the ethanol extract of MP stem:

$$y = 2.1669x + 1.5666 \quad (1)$$

Regression equation (1) achieved a value $R^2$=0.9824, so that the R calculated value was 0.9912. Based on the table value of R person, i.e., (R=0.666), which means the calculated R value was greater than the value from the R table. This indicated a significant correlation or relationship between the concentration and the percentage of shrimp larval death when the ethanol extract of MP stem was administered (Figure 3). In the probit analysis, the ethanol extract was found to be toxic at $LC_{50}$=38.41 µg/mL (Table 5). The following regression equation was obtained to calculate the toxicity of the ethyl acetate extract of MP stem:

$$y = 1.8692x + 2.155 \quad (2)$$

In regression equation (2), $R^2$=0.9646 and R count =0.9821 were obtained. The R-value of the R person table was (R=0.666), indicating that R counts were greater than those of the R table. There was a significant correlation or relationship between the concentration of the ethyl acetate extract of MP stem and the percentage of shrimp larval death (Figure 3). The probit analysis revealed that ethyl acetate extract was toxic at $LC_{50}$=33.27 µg/mL (Table 6).

Whenever the results were compared to toxicity level criteria, the crude extract at $LC_{50}$<100 µg/mL was in the strong toxicity category, and crude extracts in the range of $LC_{50}$ values of 100<$LC_{50}$< 500 µg/ml were classified as having moderate toxicity. The crude extracts with the range of $LC_{50}$ values for 500<$LC_{50}$<1000 g/ml were considered to have low toxicity activity, while those with $LC_{50}$ values greater than 1000 µg/mL were considered non-toxic.

***Cytotoxicity Assay of MP Stem Extracts to hOSCC Cells***

The cytotoxicity test results of MP stem sap deposited in a hOSCC (OM-1) cell culture revealed differences in the formazan formed in each group. Purplish crystal formation was observed in the reaction of MTT with the succinic dehydrogenase enzyme found in mitochondria (Figure 4). Formazan formed in each well was observed using an ELISA reader at a wavelength of 650 nm. The concentration inhibition of the ethanol extract of MP stem on hOSCC cells was 15.00 µg/mL ($IC_{50}$=15.00µg/mL). The inhibition of hOSCC cells was measured, and a concentration-response relationship was determined using a regression analysis. The following equation was used to calculate the concentration inhibition of the ethanol extract of MP stem against hOSCC culture:

$$y = -52.20x + 104.2 \quad (3)$$

Regression equation (3) obtained the value $R^2$=0.8769, so that the calculated value was R=0.9364. Based on the table value of R person, i.e., (R=0.344), this means that the calculated R value was greater than the value from the R table. This indicates a significant correlation or relationship between the concentrations of the ethanol extract on inhibition of cancer cell

(hOSCC) proliferation (Figure 5A). However, the 50% inhibitory concentration ($IC_{50}$) of ethyl acetate extract was 10.61 µg/mL. The regression equation of the ethyl acetate extract was as follows:

$$y = -49.90x + 98.25 \quad (4)$$

Regression equation (4) obtained an $R^2$ value of 0.9321, so that the calculated R value was 0.9654. Based on the table value of R person I.e., (R=0.344), this means that the calculated R value was greater than the value from the R table. This indicates a significant correlation or relationship between the concentrations of the ethyl acetate extract on inhibition of cancer cell (hOSCC) proliferation (Figure 5B).

***Apoptotic Activity of Caspase-3 on hOSCC Culture***

The time of incubation and concentration of MP stem ethanol extract had an influence on the concentration of caspase-3 in hOSCC culture. Whenever the MP stem ethanol extract was administered, the concentration of caspase-3 steadily increased after 2-, 24-, and 48-hours, as determined by ELISA. The Figure 6 revealed that administering an ethanol extract of MP stem at a dose of $4IC_{50}$ significantly increased caspase-3 levels compared to doses of $0.5IC_{50}$, $IC_{50}$, $2IC_{50}$, and $8IC_{50}$. However, after 2-, 24-, and 48-hours incubation, the quantity of caspase-3 was lower at the $8IC_{50}$ dose than at the $IC_{50}$, $2IC_{50}$, and $4IC_{50}$ doses.

**Discussions**

Recent anticancer drug research focused on the genetic side with a fundamental goal in mind while avoiding damaging healthy cells[35]. Natural drugs can be an alternative to chemo- and radiotherapy[36]. One of the mechanisms used in the development of anticancer drugs is the *p53* gene pathway. The *p53* gene is a tumor-suppressor gene that accumulates when DNA is damaged[37]. This gene serves as a controller of damaged cells dividing by inhibiting the cell cycle in the interphase phase or at G1[38]. Excessive cell stressors are captured by Toll-like receptors (TLRs), which then phosphorylate nuclear factor (NF)-KB and initiate the *p53* gene to perform apoptosis[39]. If stress cannot be eliminated by a cell, the *p53* gene mutates, and there is a failure of the process of apoptosis. Failure of apoptosis allows damaged cells to continue to replicate and eventually form cancer[40,41]. Caspases are a type of proapoptotic protease that cleaves key sites for apoptosis execution. Caspase-3 is an important member of this family that causes apoptosis by inducing nuclear alterations. Many forms of cancer, such as cervical and breast cancer, have reduced caspase-3 levels, whereas elevated caspase-3 levels are a good predictive factor for gastric malignancies[42].

Several chemically bioactive compounds were found in the ethanol extract, including hexadecanoic acid, methyl ester; n-hexadecanoic acid; pentadecanoic acid, 14-methyl, methyl ester; heptadecanoic acid, 16-methyl, methyl ester; and 9,12-octadecadienoic acid, which were palmitic acid and stearate acid group compounds (Table 1). Palmitic acid, stearate acid, oleic acid and linoleic acid group compounds were also found in the ethyl acetate extract, although at lower concentrations (Table 2). In a previous study, members of the palmitic acid group contained a saturated long-chain fatty acid with a 16-carbon backbone that was shown to play a role in cancer suppression, particularly in colon cancer[43].

The concentration of hexadecanoic acid methyl ester had the highest percentage (5.40%) in the ethanol extract, while 9.12-octadecadienoic acid was 11.47% according to the results of the GC-MS analysis of the ethanol extract and ethyl acetate extract of MP stem. A previous study found that hexadecanoic acid compounds were a potential source of anti-inflammatory agents that selectively induced $G_2$/M arrest and apoptosis in MCF-7 cells via upregulation of p53 and the Bax/Bcl-2 ratio[44,45], cytotoxicity against human leukemic cells, and inhibition

phagocytic activity and nitric oxide production of certain cells[46], and could lower levels of TNF-α, prostaglandin E (PGE)-2, and interleukin (IL)-10 without affecting ATP levels[47,48]. constrast, octadec-9-enoic acid and oleic acid had the potential to reduce inflammation, aid digestion, and promote probiotics and pro-omega 3[49]. It is possible that either of these identified compounds or other unknown compounds, or a synergistic effect of all these compounds combined contributed to the excellent anticancer and anti-inflammatory activity. The role of inflammation in cancer initiation and progression is well understood, and the underlying molecular mechanisms have been extensively studied. As a result, it has become feasible to target inflammatory pathways for cancer prevention and therapy in recent decades. DNA-repair proteins, caspases, lipid peroxidation, mutation, and NF-KB activation all contribute to a wide range of diseases, including cancer[50].

The PASS SERVER is commonly used to examine a compound's biological activity prior to chemical synthesis and biological testing. This method predicts the highest values of bioactive compounds based on a comparison of the Pa (probable active): Pi (probable inactive) ratio and the accuracy of the prediction was 95% based on a leave-one-out cross-validation (LOO CV) assessment[17,19]. This study found that hexadecanoic acid had a Pa=0.914 and Pi=0.003 values in the pathway via SOD inhibitors. In normal cells that experience oxidative stress, antioxidants in the body, such as SOD, dampen reactive oxygen species (ROS)[51]. High ROS and low SOD activities allow cell damage to continue, which can trigger DNA damage. Cells with damaged DNA can activate repair mechanisms through the apoptosis pathway by p53[50]. If cells with DNA damage are not immediately repaired, those cells may develop into abnormal cells (cancer). Nevertheless, antioxidant activity should be suppressed from another perspective. Some anticancer treatments, such as xenobiotics and radiation, kill tumor cells by releasing ROS. Cell with high amounts of such antioxidant enzymes are resistant to anticancer drugs[52]. The overexpression of SOD was associated with an increased incidence of tumor metastasis[53,54]. That was in accordance with this study in which potential compounds with the highest activity as agonists against apoptosis were linoleic acid (Pa=0.545) and a caspase-3/caspase-8 stimulant (Pa=0.617). Thus, the value of the Pa>Pi ratio indicated that it had the potential to be a good anticancer compound, because analysis of it using the CLC-Pred tools for in silico predictions of cell line cytotoxicity revealed that hexadecanoic acid exhibited toxicity to some cancer cell cultures such as melanomas. Therefore, MP stem extract might well inhibit the development of oral cancer (OSCC) due to its ability to induce apoptotis pathways.

Relying on the absorption computing system, distribution, metabolism, excretion, and toxicity (ADMET) screening, hexadecanoic acid or palmitic acid compounds could be well absorbed in the human intestinal tract (Pa=0.8417) because palmitic acid is a fatty acid that is highly fat-soluble, easily penetrates cell membranes, and was found in the subcellular milieu in mitochondria. This indicates that the ethanol and ethyl acetate extracts of banana stems could be orally administered.

*In vitro* testing should be used to validate the GC-MS data and computational predictions made with the PASS SERVER. They were calculated using the BSLT and Finney's *probit* analysis tool, and results showed that the $LC_{50}$ value of the ethanol extract was 38.41 µg/mL, and that of the ethyl acetate extract was 33.27 µg/mL, indicating that the ethyl acetate extract was more toxic than the ethanol extract. The $LC_{50}$ values for both extracts were less than 100 µg/m, and thus may be classified as highly toxic.

The use of ethanol and ethyl acetate extracts of MP stem in the culture method suppressed the growth of oral cancer (hOSCC) cells. The concentration of ethyl acetate extract that inhibited the proliferation of oral cells was lower than that of the ethanol extract. This was consistent with the wide range of chemicals discovered using the GC-MS technique on the ethyl acetate extract. As a result, when utilizing the ethanol extract to prevent cancer cell

proliferation, a larger concentration will be necessary than when using the ethyl acetate extract. In the ethyl acetate extract, we found 15 chemically active compounds, and we found seven in the ethanol extract. The active compound of octadec-9-enoic acid and oleic acid which had potential as pro-omega 3. This would contribute to be a good anticancer.

In this study, apoptotic activity was observed by measuring the concentration of caspase-3 in hOSCC cell cultures treated with ethanol extract. The screening results through the GC-MS method showed that the concentration of palmitic acid derivative compounds, especially hexadecanoic acid, was higher in ethanol extracts. Compounds of hexadecanoic acid are predicted to have anticancer activity in several types of cancer, one of which is through the stimulation of caspase-3 activity. An increase in the concentration of caspase-3 in the application of ethanol extract of MP stem showed an increase in the apoptotic activity of cancer cells. The content of hexadecanoic acid can inhibit the growth of oral cavity cancer cells (hOSCC) according to the dose. There is a dose relationship with the administration of ethanol extract of MP stem at doses of $0.5IC_{50}$, $IC_{50}$, $2IC_{50}$, and $4IC_{50}$ to increase apoptotic activity by measuring caspase-3. However, there was a decrease in apoptotic activity with a hefty dose of $8IC_{50}$ (360mg/ml). A high-dose administration of MP stem ethanol extract in hOSCC cell cultures could inhibit apoptotic activity, which was indicated by low concentrations of caspase-3. As a result, the dose- and time-dependence of drug-induced apoptosis should be thoroughly assessed for both experimental and conventional anticancer agents.

## Conclusions

We concluded that the chemically active compounds such as hexadecanoic acid obtained from the *Musa paradisiaca var. sapientum (L) Kunz* (MP) stem could be developed as candidates for new anticancer drugs. Hexadecanoic acid is palmitic acid derivates of MP stem extract has apoptotic activity by inhibiting cancer cell growth of hOSCC culture through caspase-3 stimulants.


## Acknowledgments

We'd like to express our gratitude to Universitas Airlangga for supporting research laboratories in conducting this study.

## Funding Statement

The Ministry of Research and Technology /National Agency for Research and Innovation of Republic Indonesia on Project Number: 308/UN3.15/PT/2021.


## Authors' Contributions

Conceptualization, H.S.B. and Y.K.S.; methodology, H.S.B. and Y.K.S.; investigation and data curation, N.M.U. and M.A.S.; data analysis, R.R.; resources, H.S.B.; writing-original draft preparation, H.S.B. and S.A.; writing-review and editing, supervision, H.S.B. Y.K.S. and C.Z.W. All authors contributed to the final version and approved the manuscript.

## Competing Interests

We have no competing interests.

## Ethical Statement

Ethics approval was not required for this study.

## Data Availability Statement

The data used to support the finding of this study are included within the article.

**References**


1. Saraswat N, Pillay R, Everett B, George A. Knowledge, attitudes and practices of South Asian immigrants in developed countries regarding oral cancer: An integrative review. BMC Cancer 2020; 20: 477-493. doi:10.1186/s12885-020-06944-9
2. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The prevalence of squamous cell carcinoma in different sites of oral cavity at our rural health care centre in Loni, Maharashtra – a retrospective 10-year study. Contemp Oncol (Pozn) 2017; 2: 178-183. doi:10.5114/wo.2017.68628
3. Bugshan A, Farooq I. Oral squamous cell carcinoma: Metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res 2020; 9: 229. doi:10.12688/f1000research.22941.1
4. Miranda-Filho A, Bray F. Global patterns and trends in cancers of the lip, tongue and mouth. Oral Oncol 2020; 102: 104551. doi:10.1016/j.oraloncology.2019.104551
5. Ye L, Jiang Y, Liu W, Tao H. Correlation between periodontal disease and oral cancer risk: A meta-Analysis. J. Cancer Res Ther 2016; 12: 237-240. doi:10.4103/0973-1482.200746
6. Chen YH, Zou XN, Zheng TZ, Zhou Q, Qiu H, Chen YL, He M, Du J, Lei HK, Zhao P. High spicy food intake and risk of cancer: A meta-analysis of case–control studies. Chin Med J 2017; 130: 2241–2250. doi:10.4103/0366-6999.213968
7. Farah CS, Kujan O, Prime S, Zain RB. Oral mucosal malignancies chapter. Contemporary Oral Medicine. Springer Cham, 2019. doi:10.1007/978-3-319-72303-7_21
8. Zhang HH, Zhang Y, Cheng YN. Metformin in combination with curcumin inhibits the growth, metastasis, and angiogenesis of hepatocellular carcinoma in vitro and in vivo. Mol Carcinog 2018; 57: 44-56. doi:10.1002/mc.22718
9. Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis 2019; 10: 177-189. doi:10.1038/s41419-019-1407-6
10. Li W, Jiang Z, Xiao X. Curcumin inhibits superoxide dismutase-induced epithelial-to-mesenchymal transition via the PI3K/Akt/NF-κB pathway in pancreatic cancer cells. Int J Oncol 2018; 52: 1593-1602. doi:10.3892/ijo.2018.4295
11. Güçlü H, Doganlar ZB, Gürlü VP, Özal A, Dogan A, Turhan MA, Doganlar O. Effects of cisplatin-5-fluorouracil combination therapy on oxidative stress, DNA damage, mitochondrial apoptosis, and death receptor signalling in retinal pigment epithelium cells. Cutan Ocul Toxicol 2018; 37: 291-304. doi:10.1080/15569527.2018.1456548
12. Celentano A, McCullough M, Cirillo N. Glucocorticoids reduce chemotherapeutic effectiveness on OSCC cells via glucose-dependent mechanisms. J Cell Physiol 2019; 234: 2013-2020. doi:10.1002/jcp.27227
13. Kaae JK, Stenfeldt L, Eriksen JG. Xerostomia after radiotherapy for oral and oropharyngeal cancer: Increasing salivary flow with tasteless sugar-free chewing gum. Front Oncol 2016; 6: 111-116. doi:10.3389/fonc.2016.00111
14. Al-Rubaye AF, Hameed IH, Kadhim M. A review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. Int J Toxicol Pharmacol Res 2017; 9: 948-953. doi:10.25258/ijtpr.v9i01.9042
15. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: A review. Futur J Pharm Sci 2020; 6: 67. doi:10.1186/s43094-020-00091-5
16. Zhang A, Sun H, Wang X. Mass spectrometry-driven drug discovery for development of herbal medicine. Mass Spectrom Rev 2018; 37: 307-320. doi:10.1002/mas.21529
17. Rudik A, Dmitriev A, Lagunin A, Filimonov D, Poroikov V. Metapass: A web application for analyzing the biological activity spectrum of organic compounds taking into account their biotransformation. Mol Inf 2021; 40: 103-110. doi:10.1002/minf.202000231
18. Chinnasamy P, Arumugam R. In silico prediction of anticarcinogenic bioactivities of traditional anti-inflammatory plants used by tribal healers in sathyamangalam wildlife

Sanctuary, India. Egypt J Basic Appl Sci 2018; 5: 265-279. doi:10.1016/j.ejbas.2018.10.002
19. Filimonov DA, Rudik AV, Dmitriev AV, Poroikov VV. Computer-aided estimation of biological activity profiles of drug-like compounds taking into account their metabolism in human body. Int J Mol Sci 2020; 21: 7492. doi:10.3390/ijms21207492
20. Lagunin AA, Dubovskaja VI, Rudik AV, Pogodin PV, Druzhilovskiy DS, Gloriozova TA, Filimonov DA, Sastry NG, Poroikov VV. CLC-Pred: A freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. PLoS ONE 2018; 13: e0191838. doi:10.1371/journal.pone.0191838
21. Finney D. Probit analysis. J Pharm Sci 1971; 60: 1432. doi:10.1002/jps.2600600940
22. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982; 45: 31-34. doi:10.1055/s-2007-971236
23. Matata DZ, Ngassapa OD, Machumi F, Moshi MJ. Screening of plants used as traditional anticancer remedies in mkuranga and same districts, tanzania, using brine shrimp toxicity bioassay. Evid Based Complement Alternat Med 2018; 2018: 3034612. doi:10.1155/2018/3034612
24. Du L, Ma S, Wen X, Chai J, Zhou D. Oral squamous cell carcinoma cells are resistant to doxorubicin through upregulation of miR-221. Mol Med Rep 2017; 16: 2659-2667. doi:10.3892/mmr.2017.6915
25. Chaudhary S, Chandrashekar KS, Pai KS, Setty MM, Devkar RA, Reddy ND, Shoja MH. Evaluation of antioxidant and anticancer activity of extract and fractions of Nardostachys jatamansi DC in breast carcinoma. BMC Complement Altern Med 2015; 15: 50. doi:10.1186/s12906-015-0563-1
26. Jyothirmayi N, Rao M. Banana medicinal uses. J Med Sci Tech 2015; 4: 152-160.
27. Sudirga S. Utilization of plants as traditional medicines in Trunyan village, Kintamani district, Bangli regency. J Bumi Lestari Udayana 2000; 12: 7-18.
28. Budi HS, Astuti ER. The MMP-2, MMP-9 expression and collagen density of the ambonese banana stem sap administration on wound healing. J Int Dent Med Res 2019; 12: 492-497.
29. Budi HS, Soesilowati P, Imanina Z. Gambaran histopatologi penyembuhkan luka pencabutan gigi pada makrofag dan neovaskular dengan pemberian getah batang pisang ambon. Maj Ked Gigi Indones 2017; 3: 121-127. doi:10.22146/majkedgiind.17454
30. Deng W, Peng W, Wang T, Chen J, Zhu S. Overexpression of MMPs functions as a prognostic biomarker for oral cancer patients: A systematic review and meta-analysis. Oral Health Prev Dent 2019; 17: 505-514. doi:10.3290/j.ohpd.a43636
31. Huang H. Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: Recent advances. Sensors 2018; 18: 3249. doi:10.3390/s18103249
32. Azmin SNHM, Manan ZA, Alwi SRW, Chua LS, Mustaffa A, Yunus NA. Herbal processing and extraction technologies. Sep Purif Rev 2016; 45: 305-320. doi:10.1080/15422119.2016.1145395
33. Budi HS, Juliastuti WS, Ariani W. MTT-based cytotoxic evaluation of ambonese banana stem sap (Musa paradisiaca var. Sapientum (L.) Kuntze) on fibroblast cells. Period Tche Quim 2020; 17: 558-564. doi:10.52571/PTQ.v17.n36.2020.639_Periodico36_pgs_624_633.pdf
34. Freshney RI. Culture of animal cells: A manual of basic technique and specialized applications. Wiley-Blackwell, 2016.
35. Maeda H, Khatami M. Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. Clin Transl Med 2018; 7: 11-30. doi:10.1186/s40169-018-0185-6

36. Zhang QY, Wang FX, Jia KK, Kong LD. Natural product interventions for chemotherapy and radiotherapy-induced side effects. Front Pharmacol 2018; 9: 1253. doi:10.3389/fphar.2018.01253
37. Misra A, Rai S, Misra D. Functional role of apoptosis in oral diseases: An update. J Oral Maxillofac Pathol 2016; 20: 491-496. doi:10.4103/0973-029X.190953
38. Williams AB, Schumacher B. P53 in the DNA-damage-repair process. Cold Spring Harb Perspect Med 2016; 6: 1-16. doi:10.1101/cshperspect.a026070
39. Chen J. The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. Cold Spring Harb Perspect Med 2016; 6: 1-16. doi:10.1101/cshperspect.a026104
40. Rowan S, Ludwig RL, Haupt Y, Bates S, Lu X, Oren M, Vousden KH. Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. EMBO J 1996; 15: 827-838.
41. Schlereth K, Beinoraviciute-Kellner R, Zeitlinger MK, Bretz AC, Sauer M, Charles JP, Vogiatzi F, Leich E, Samans B, Eilers M, Kisker C, Rosenwald A, Stiewe T. DNA binding cooperativity of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol Cell 2010; 38: 356-368. doi:10.1016/j.molcel.2010.02.037
42. Xu M, Xia LP, Fan LJ, Xue JL, Shao WW, Xu D. Livin and caspase-3 expression are negatively correlated in cervical squamous cell cancer. Eur J Gynaecol Oncol 2013; 34: 152-155.
43. Arun KB, Madhavan A, Reshmitha TR, Thomas S, Nisha P. Musa paradisiaca inflorescence induces human colon cancer cell death by modulating cascades of transcriptional events. Food Funct 2018; 9: 511-524. doi:10.1039/c7fo01454f
44. Ghate NB, Das A, Chaudhuri D, Panja S, Mandal N. Sundew plant, a potential source of anti-inflammatory agents, selectively induces G2/M arrest and apoptosis in MCF-7 cells through upregulation of p53 and Bax/Bcl-2 ratio. Cell Death Discov 2016; 2: 15062. doi:10.1038/cddiscovery.2015.62
45. Achakzai JK, Anwar Panezai M, Kakar MA, Kakar AM, Kakar S, Khan J, Khan NY, Khilji I, Tareen AK. In vitro anticancer MCF-7, anti-inflammatory, and brine shrimp loethality assay (BSLA) and GC-MS analysis of whole plant butanol fraction of rheum ribes (WBFRR). BioMed Res Int 2019; 2019: 3264846. doi:10.1155/2019/3264846
46. Mellado M, Soto M, Madrid A, Montenegro I, Jara-Gutiérrez C, Villena J, Werner E, Godoy P, Aguilar LF. In vitro antioxidant and antiproliferative effect of the extracts of Ephedra chilensis K Presl aerial parts. BMC Complement Altern Med 2019; 19: 53. doi:10.1186/s12906-019-2462-3
47. Mericli F, Becer E, Kabadayı H, Hanoglu A, Yigit Hanoglu D, Ozkum Yavuz D, Ozek T, Vatansever S. Fatty acid composition and anticancer activity in colon carcinoma cell lines of Prunus dulcis seed oil. Pharm Biol 2017; 55: 1239-1248. doi:10.1080/13880209.2017.1296003
48. Lin HW, Liu CZ, Cao D, Chen PY, Chen MF, Lin SZ, Mozayan M, Chen AF, Premkumar LS, Torry DS, Lee TJ. Endogenous methyl palmitate modulates nicotinic receptor-mediated transmission in the superior cervical ganglion. Proc Natl Acad Sci USA 2008; 105: 19526-19531. doi:10.1073/pnas.0810262105
49. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res 2002; 22: 2587-2590.
50. Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biol 2019; 25: 101084. doi:10.1016/j.redox

51. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: Harms and benefits for human health. Oxid Med Cell Longev 2017; 2017: 8416763. doi:10.1155/2017/8416763
52. Ma X, Deng D, Chen W. Inhibitors and activators of SOD, GSH-Px, and CAT: Chapter. Enzyme inhibitors and activators. Intechopen, 2017. doi:10.5772/65936
53. Griess B, Tom E, Domann F, Teoh-Fitzgerald M. Extracellular superoxide dismutase and its role in cancer. Free Radic Biol Med 2017; 112: 464-479. doi:10.1016/j.freeradbiomed.2017.08.013
54. Che M, Wang R, Li X, Wang HY, Zheng XFS. Expanding roles of superoxide dismutases in cell regulation and cancer. Drug Discov Today 2016; 21; 143-149. doi:10.1016/j.drudis.2015.10.001

## Table captions

**Table 1.** GC-MS analysis of chemically active compounds in *Musa paradisiaca var. sapientum (L) Kunz* ethanol extract

| Peak | Retention time | Peak area (%) | Chemically active compounds | Qual |
|---|---|---|---|---|
| 1 | 15.815 | 5.40 | Hexadecanoic acid, methyl ester | 98 |
| | | | Pentadecanoic acid, 14-methyl-, methyl ester | 97 |
| 2 | 15.996 | 3.85 | n- Hexadecanoic acid | 95 |
| 3 | 16.930 | 2.29 | Methyl stearate | 91 |
| | | | Heptadecanoic acid, 16-methyl-, methyl ester | 91 |
| | | | Heptadecanoic acid, 14-methyl-, methyl ester | 90 |
| 4 | 17.024 | 11.47 | 9,12-Octadecadienoic acid | 46 |
| 5 | 17.101 | 0.96 | Benzo (h) quinoline, 2,4-dimethyl | 38 |

**Table 2.** GC-MS analysis of chemically active compounds in *Musa paradisiaca var. sapientum (L) Kunz* ethyl acetate extract

| Peak | Retention time | Peak area (%) | Chemically active compounds | Qual |
|---|---|---|---|---|
| 1 | 14.924 | 0.28 | Cis-2,4,5-Trimethoxy-.beta.-methyl-.beta.-nitrostyrene | 38 |
| 2 | 15.055 | 0.14 | Triamterene | 41 |
| 3 | 15.224 | 0.25 | 4-Fluorobenzoic acid, 2-phenylethyl ester | 27 |
| 4 | 15.815 | 1.73 | Pentadecanoic acid, 14-methyl-, methyl ester | 99 |
| | | | Hexadecanoic acid, methyl ester | |
| 5 | 15.997 | 1.33 | n- Hexadecanoic acid | 99 |
| 6 | 16.768 | 0.39 | Methyl 10-trans, 12-cis-octadecadienoate | 99 |
| | | | 9,12-Octadecadienoic acid (z,z)-, methyl ester | 99 |
| | | | 9,15-Octadecadienoic acid (z,z)-, methyl ester | 99 |
| 7 | 16.796 | 0.68 | 9-Octadecadienoic acid (z)-, methyl ester | 99 |
| | | | Cis-13-Octadecadienoic acid, methyl ester | 99 |
| 8 | 16.931 | 0.98 | Methyl stearate | 98 |
| 9 | 17.021 | 4.62 | Oleic acid | 96 |
| | | | Octadec-9-enoic acid | 96 |
| 10 | 17.145 | 2.08 | 9,12-Octadecadienoic acid (z,z)-, Oleic acid | 95 |
| 11 | 17.788 | 0.20 | Cyclopropaneoctanal, 2-octyl- | 90 |
| 12 | 18.850 | 0.81 | 3'-Chlorooxanilic acid N'(3-ethoxy-4-hydroxybenzylidene) hydrazide | 42 |
| 13 | 19.065 | 1.21 | Ethanol, 2-bromo- | 35 |
| 14 | 19.432 | 0.44 | 2-propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-3 | 64 |
| 15 | 19.993 | 0.19 | Oleic acid | 47 |

**Table 3.** *Canonical smile* of fatty acid compounds group has anticancer potential in *Musa paradisiaca var. sapientum (L) Kunz*

| No | Chemically active compounds | Synonym | Canonical SMILE | Pa value | Activity |
|---|---|---|---|---|---|
| 1 | Hexadec-anoic acid, methyl ester | Palmitic acid methyl ester<br>Methyl palmitate | CCCCCCCCCCCCCCCC(=O)OC | 0.738 | Superoxide dismutase inhibitor |
| | | | | 0.473 | Apoptosis agonist |
| | | | | 0.609 | Caspase 3 and 8 stimulant |
| 2 | Pentade-canoic acid, 14-methyl-, methyl ester | Pentadecanoic acid<br>Pentadecylic acid<br>1002-84-2<br>n-Pentadecanoic acid<br>Pentadecyclic acid | CCCCCCCCCCCCCCC(=O)O | 0.914 | Superoxide dismutase inhibitor |
| | | | | 0.342 | Apoptosis agonist |
| | | | | 0.562 | Caspase 3 and 8 stimulant |
| 3 | n- Hexadec-anoic acid | Palmitic acid<br>Hexadecanoic acid<br>57-10-3<br>Cetylic acid<br>Palmitate | CCCCCCCCCCCCCCCC(=O)O | 0.914 | Superoxide dismutase inhibitor |
| | | | | 0.342 | Apoptosis agonist |
| | | | | 0.562 | Caspase 3 and 8 stimulant |
| 4 | Methyl stearate | Methyl octadecanoate<br>112-61-8<br>Octadecanoic acid, methyl ester<br>Stearic acid methyl ester | CCCCCCCCCCCCCCCCCC(=O)OC | 0.738 | Superoxide dismutase inhibitor |
| | | | | 0.336 | Apoptosis agonist |
| | | | | 0.609 | Caspase 3 and 8 stimulant |
| 5 | Heptade-canoic acid, 16-methyl-, methyl ester | Methyl heptadecanoate<br>1731-92-6<br>Methyl margarate<br>Heptadecanoic acid, methyl ester<br>Margaric acid methyl ester | CCCCCCCCCCCCCCCCC(=O)OC | 0.738 | Superoxide dismutase inhibitor |
| | | | | 0.473 | Apoptosis agonist |
| | | | | 0.609 | Caspase 3 and 8 stimulant |
| 6 | 9,12-Octadec-adienoic acid (z,z)-, methyl ester<br>Cis-13-Octadecadieno ic acid, methyl ester | Linoleic acid<br>60-33-3<br>Linolic acid<br>Telfairic acid<br>cis,cis-Linoleic acid | CCCCCC=CCC=CCCCCCCCC(=O)O | 0.814 | Superoxide dismutase inhibitor |
| | | | | 0.545 | Apoptosis agonist |
| | | | | 0.617 | Caspase 3 and 8 stimulant |
| 7 | Oleic acid<br>Octadec-9-enoic acid | oleic acid<br>112-80-1<br>cis-9-Octadecenoic acid<br>(Z)-Octadec-9-enoic acid<br>cis-Oleic acid | CCCCCCCCC=CCCCCCCCC(=O)O | 0.851 | Superoxide dismutase inhibitor |
| | | | | 0.499 | Apoptosis agonist |
| | | | | 0.592 | Caspase 3 and 8 stimulant |
| 8 | | | CCCCCCCCC1CC1CCCCCCCC=O | 0.653 | Superoxide dismutase inhibitor |

| | | | | | |
|---|---|---|---|---|---|
| | Cyclopr-opaneoctanal, 2-octyl- | 2-Octylcyclopropaneoctanal<br>8-(2-Octylcyclopropyl)octanal<br>2-octyl-cyclopropaneoctanal | | 0.386 | Apoptosis agonist |
| | | | | 0.411 | Caspase 3 and 8 stimulant |

**Table 4.** Toxicity of *hexadecanoic acid* on cell cancer culture used the *CLC- Pred tools*

| Pa | Pi | Cell-line | Cell-line full name | Tissue | Tumor type |
|---|---|---|---|---|---|
| 0.558 | 0.017 | DMS-114 | Lung carcinoma | Lung | Carcinoma |
| 0.545 | 0.017 | SK-MEL-1 | Metastatic melanoma | Skin | Melanoma |
| 0.521 | 0.009 | A2058 | Melanoma | Skin | Melanoma |
| 0.537 | 0.033 | NCI-H838 | Non-small cell lung cancer (stage 3) | Lung | Carcinoma |

**Table 5.** *Probit* analysis of Brine Shrimp Lethality Test on *Musa paradisiaca var. sapientum (L) Kunz* ethanol extract

| Concentration | Log concentration | Life | Mortality | Probit | $LC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 10 | 1 | 9 | 10% | 3.72 | 38.41276 |
| 20 | 1.30103 | 7 | 30% | 4.48 | |
| 30 | 1.477121 | 6 | 40% | 4.75 | |
| 40 | 1.60206 | 5 | 50% | 5.00 | |
| 50 | 1.69897 | 4 | 60% | 5.25 | |
| 60 | 1.778151 | 4 | 60% | 5.25 | |
| 70 | 1.845098 | 3 | 70% | 5.52 | |
| 80 | 1.90309 | 2 | 80% | 5.84 | |
| 90 | 1.954243 | 2 | 80% | 5.84 | |

**Table 6.** *Probit* analysis of *Musa paradisiaca var. sapientum (L) Kunz* on ethyl acetate extract

| Concentration | Log concentration | Life | Mortality | Probit | $LC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 10 | 1 | 8 | 20% | 4.16 | 33.26914 |
| 20 | 1.30103 | 7 | 30% | 4.48 | |
| 30 | 1.477121 | 6 | 40% | 4.75 | |
| 40 | 1.60206 | 4 | 60% | 5.25 | |
| 50 | 1.69897 | 4 | 60% | 5.25 | |
| 60 | 1.778151 | 3 | 70% | 5.52 | |
| 70 | 1.845098 | 3 | 70% | 5.52 | |
| 80 | 1.90309 | 2 | 80% | 5.84 | |
| 90 | 1.954243 | 2 | 80% | 5.84 | |

**Figure captions**

**Figure 1.** GC-MS chromatogram of MP stem ethanol extract.
**Figure 2.** GC-MS chromatogram MP stem ethyl acetate extract.
**Figure 3.** Correlation between extract concentration and Brine Shrimp Lethality Test. The orange line represented MP stem ethanol extract, and the blue line represented MP stem ethyl acetate extract.
**Figure 4.** Cytotoxicity assay of hOSCC culture by MTT method. A. Seeding cell, B. Harvesting cell, and C. Formazan formation.
**Figure 5.** Inhibition concentration of MP stem extract to hOSCC culture. A. $IC_{50}$ of ethanol extract and B. $IC_{50}$ of ethyl acetate extract
**Figure 6.** Caspase-3 level on hOSCC culture treated MP stem ethanol extract. A. 96 well plate scheme, B. Concentration of caspase-3 in different doses and incubation time. Conc.: concentration.

# Your article #27067 is Revised

**Publishing Manager** <info@publishingmanager.org>
Reply-To: "info@publishingmanager.org" <info@publishingmanager.org>
To: hendrik-s-b@fkg.unair.ac.id

Wed, Aug 24, 2022 at 3:58 PM

Dear Doctor,

below the request to continue the review process of the manuscript **#27067** entitled "Palmitic acid of Musa Paradisiaca induce apoptosis through caspase-3 in human oral squamous cell carcinoma".

**Request:** Dear Dr, the Peer review has been successful; nevertheless, because of a technical issue occurred on the website, we are no more able to download your previously uploaded files and media (manuscript, tables and figures, copyright form, cover letter, etc.). To let us proceed with the comments, please kindly upload your files again. Thank you for Your understanding..

Best regards,
Journal Editorial Team

# Article #27067 submitted

**Publishing Manager** <info@publishingmanager.org>
Reply-To: "info@publishingmanager.org" <info@publishingmanager.org>
To: hendrik-s-b@fkg.unair.ac.id

Wed, Aug 31, 2022 at 4:29 PM

Dear Doctor,

Thank you for submitting your article "Palmitic acid of Musa Paradisiaca induce apoptosis through caspase-3 in human oral squamous cell carcinoma" to European Review for Medical and Pharmacological Sciences.

We are in the process of evaluating your manuscript. We evaluate all manuscript submissions as expeditiously as possible and appreciate your patience throughout the peer-review process.

Best regards,
Journal Editorial Team

Dear Editor

Thank you for giving us the opportunity to submit a revised draft of the manuscript "**Palmitic acid of *Musa Paradisiaca* induce apoptosis through caspase-3 in human oral squamous cell carcinoma**" for publication in the "**European Review for Medical and Pharmacological Sciences**".

We appreciate the time and effort that you and the reviewers dedicated to providing feedback on our manuscript and are grateful for the insightful comments on and valuable improvements to our paper. We have incorporated most of the suggestions made by the reviewers. Those changes are highlighted within the manuscript in blue. Please see in the attachment files, for a point-by-point response to the reviewers' comments and concerns.

Thank you very much for your kindness.

Assoc. Prof. Dr. Hendrik Setia Budi, DDS, MDS
Corresponding author

**Response to Reviewer**

**Comment 1:**

Please provide the limitations of the study and create a specific paragraph at the end of the discussion's section

**Response:**

Thank you for your response and reviews. We have added the limitation of this study in this manuscript.

**Comment-2:**

In the discussion chapter, please include more recent published articles.

**Response:**

Thank you for your response and reviews. We have provided the recent published articles in the discussion section.

**Comment-3:**

Moreover, please note that the paper contains many typos and grammatical mistakes. You are therefore encouraged to proof-read thoroughly the text, check any double/missing space and improve English before resubmission

**Response:**

Thank you for your response and reviews. We have proofread and revised it in the manuscript with concern

**Response to Editorial**

**Comments:**

• Please add a comma in numbers > 1000 (not 1868 but 1,868).

• Please write “Table 1,2,3” as “Table I, II, III” and so on, both in the text and tables.

• If applicable, please provide the statistical significance of the p-value.

• In the final references, please remove the doi address.

• Check our recent published articles on the topic (see www.europeanreview.org) and see if you can find some interesting articles to improve your work.

**Response:**

Thank you for your response and reviews. We have revised it point by point in the manuscript with concern.

**Abstract**

**Objective:** Despite apoptosis processes being conserved, cancer cells have developed mechanisms to inhibit apoptosis by altering anti apoptotic molecules or inactivating pro-apoptotic. The aim of this study was to determine the palmitic acid of *Musa paradisiaca var. sapientum (L) Kunz* (MP) stem extracts against human oral squamous cell carcinoma (hOSCC) through caspase-3.

**Materials and Methods:** Ethanol and ethyl acetate extracts of MP stem was analyzed by gas chromatography-mass spectrometry (GC-MS). Computerized models of chemically active compounds were used to predict anticancer activity. Cytotoxicity was evaluated in *Artemia salina Leach* and hOSCC (OM-1) culture at concentrations 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL respectively. The expression level of caspase-3 on hOSCC was measured by Elisa.

**Results:** We found seven chemically active compounds in the ethanol extract and 15 compounds in the ethyl acetate extract of MP stem. The major component was hexadecanoic acid of palmitic acid derivates, and this was predicted to have anticancer activities as apoptosis through caspase-3 stimulants. However, cytotoxicity effects against hOSCC culture were assessed by values of the 50% inhibitory concentration ($IC_{50}$) of 15.00 µg/mL for the ethanol extract, and an $IC_{50}$ of 10.61 µg/mL for the ethyl acetate. There was increasing significantly of caspase-3 level on treatment groups compared than control.

**Conclusions:** Hexadecanoic acid of MP stem extracts have anticancer activity by inhibiting cell growth of hOSCC culture through caspase-3 stimulants.

**Keywords:** oral squamous cell carcinoma; anticancer; palmitic acid; apoptosis; caspase-3

## Introduction

Oral cancer is a common cancer in both developing and developed countries when people undergo lifestyle changes[1]. One of the prominent types of oral cancer is oral squamous cell carcinoma (OSCC), which has reached a high number of cases in Southeast Asia with a prevalence of more than 90% among oral cancers[2]. OSCC occurs place in any region of the oral cavity, pharyngeal region and salivary glands[3]. It was traditionally associated with certain risk factors, such as heavy smoking, consumption of betel nut and alcohol[4], an impacted wisdom tooth, poor dental fillings, ill-fitting denture prostheses[5], unhealthy diet and nutrition[6], and the human papillomavirus (HPV) infection[7].

Irritating chemicals and free radicals as stress signals received by intercellular molecules mediate mitochondrial outer membrane permeabilization and released pro-apoptotic molecules into the cytoplasm[8]. The B-cell lymphoma (Bcl-2) family and inhibitors of apoptotic proteins that serve as apoptotic switches, control the permeabilization of mitochondrial membranes[9]. Some suggested that these two pathways may work in synchronization to initiate apoptosis. Despite apoptosis processes being conserved, cancer cells have developed mechanisms to inhibit apoptosis by altering anti apoptotic molecules or inactivating pro-apoptotic cell death components through decreasing of caspase-3. Cancer cells had adopted several mechanisms to evade apoptosis[10]. These mechanisms can be windows into the development new drugs as cancer therapy.

Chemotherapy and radiotherapy are first-line treatment regimens to manage OSCC. However, these conventional therapies have several severe side effects due to their non-specific actions against highly proliferating normal cells. It is been well known that nausea, vomiting, hair loss, Candida infections, and epithelial-ulceration are common manifestations of adverse effects derived from the use of anticancer drugs such as cisplatin, carboplatin, 5-fluorouracil[11], docetaxel, paclitaxel, and methotrexate[12], and radiation-induced xerostomia[13]. Thus, many

studies have been conducted to develop non-toxic herbal medicines as alternative to chemical drugs, with the hope of reducing severe side effects in cancer patients.

Plants contain a broad array of phytochemicals with various functional and non-functional activities, and plant extracts might be subjected to particular prescreening prior to detailed investigations. One of methods to screen the active chemical compounds in medicinal plants is through gas chromatography-mass spectrometry (GC-MS)[14]. GC-MS is a key technique to detect and separate volatile compounds, including secondary metabolites in both plant and non-plant species[15,16]. In addition to the intense laboratory-based screening and analytical processes, the emergence of computational screening that utilizes artificial intelligence systems has expedited the development of new candidates through drug discovery. Database applications such as *Prediction of Activity Spectra for Substances* (PASS SERVER), a web-based application are being used to evaluate the biological potentials of organic molecules that can be developed as medicines[17,18]. Prediction results of PASS SERVER are presented as values of Pa (probable activity) and Pi (probable inactivity), with the Pa>Pi ratio indicating a good probability of predicted activity[19]. The robustness of this technique made the screening of new anticancer drugs more feasible, and additional analytical assistance from *in silico* prediction of cell line cytotoxicity (CLC-Pred tools) adds value in predicting the toxicity to cancer cell cultures[20]. Chemically active compounds with potential anticancer activity that are sorted using GC-MS and computational (*in silico*) modeling should be tested for their biological and toxicological activities. The brine shrimp lethality test (BSLT), a bioassay that screens the cytotoxicity of a specific compound or extract, uses shrimp larvae of *Artemia salina Leach* to determine the 50% lethal concentration ($LC_{50}$) through probit analysis[21-23]. A 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) in vitro cell proliferation assay is one of the most extensively used methods for assessing preliminary anticancer activities of synthetic derivatives, natural medicines and natural product extracts. This colorimetric-based test is highly reliable and can be used on a wide range of cell lines. This assay indicates whole-cell cytotoxicity; however, additional experiments were required to define particular molecular target[24,25].

Banana trees are tropical plants that are often used as herbal medicines. The banana tree can be used as an alternative treatment of wounds, fevers, insect bites, digestive disorders, and epilepsy[26]. The sap of the Ambonese banana (*Musa paradisiaca var. sapientum (L) Kunz*) (MP) stem has long been used to accelerate wound healing and relieve tooth pain in the village community of Trunyan, Bali, Indonesia[27]. There are significant compounds in banana plants, such as saponins, flavonoids, anthraquinones, and tannins contained in both the fruit and sap, as well as lectin[28]. Sap aspirated from the stem of the MP was shown to have many therapeutic activities, including wound healing[29]. Since high levels of quercetin and lectin are found in banana sap, it was shown to suppress cancer cell proliferation, increase macrophage activity, inhibit matrix metallopreinases (MMP)-2 and -9 activities[30,31], increase T cell proliferation[28], increase fibroblast activity and promote angiogenesis[29].

In the present study, new chemically active compounds derived from the extract of MP stem were screened using GC-MS methods, *in silico* prediction activity, the in vitro BSLT and MTT assay, and also apoptotic activity of caspase-3 as potential candidates for anticancer drug.

## Materials and Methods

### *Preparation of MP Stem Extract*

Stems of MP were cleaned and chopped to a size of 0.5-1 cm. Then, the stems were dried in an oven at a temperature of 50 °C for 3 h. The dried stems were blended to become a powder form. Maceration was conducted with 96% ethanol or acetic acid as solvent for 7 days for each solvent. First, 1 kg powder of MP was mixed with 2 L of 96% ethanol and 1 kg powder was mixed with 2 L of 96% ethanol and 20 mL of glacial acetic acid. All process required 7 days

and were conducted twice for each solvent. The product was filtered through a no. 41 Whatman membrane (Merck, USA). The extract and solvent were separated by a rotary evaporator (Heidolp) at a temperature 50 °C and a speed of 200 rpm for 2 h until a condensed extract was obtained. This was placed in a covered container to avoid light[32].

***Preparation of Stock Solutions***

This study was carried out with 10-100 µg/mL as working concentrations. First, a stock solution was made by dissolving 1000 mg of MP stem extract in 1 L of sterile distilled water (1,000 mg/L=1,000 ppm), and then 1 mL of the stock solution was mixed with 9 mL of sterile distilled water (DIW) to make a concentration of 100 ppm (100 µg/mL). We then mixed 0.9 mL of the stock solution with 9.1 mL of sterile DIW to make 90 ppm (90 µg/mL). The same preparation methods were used to obtain concentrations of 80, 70, 60, 50, 40, 30, 20 and 10 µg/mL[33].

***Analysis of Chemically Active Compounds by GC-MS***

Stem extract of MP was analyzed by GC-MS (Shimadzu QP 5000, Japan). GC-MS spectroscopy was operated using a capillary column with a height of 25 cm, a diameter of 0.25 mm, and a thickness of 0.25 µm. A sample (1 µL) was injected into the mobile phase (helium gas, at a pressure of 12kPa), and it carried the sample mixture to the stationary phase (CP-Sil 5 CB). The capillary column was in an oven that was programmed to gradually increase the temperature (70 to 270 °C), at a rate of 10 °C/min. Total separation of the mixture components was 30 mL/min, and thus a chromatogram was produced representing an m/z ratio of 1:50[14].

***Predictions of Chemically Active Compounds of MP Stem Extract on PASS the SERVER***

The National Centre for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/) was used to obtain chemical structures of selected phytoconstituents of MP stem extracts. Selected structures were downloaded as sdf files for prediction in PASS SERVER software (vers. 2.0; http://www.way2drug.com/PASSonline/), and a compound's predicted activity spectrum was used to calculate the probable activity (Pa) and probable inactivity (Pi). The prediction of this spectrum by PASS SERVER was based on a structure-activity relationship (SAR) analysis of a training set containing over 205,000 compounds with over 3750 different biological activities. Pa and Pi values range 0.000-1.000 because they are probabilities. Generally, $Pa+Pi \neq 1$ since these probabilities were calculated independently[17].

***Preparation of Shrimp Larvae Culture (A. salina Leach)***

Artificial seawater was prepared. Briefly, shrimp larval eggs were dropped into an aquarium which contained NaCl (15 g) in 1 L of DIW at a neutral pH (8-9). The container placed in the room with light, and the temperature was maintained at around 25-31 °C. After 48 h the eggs hatched and the larvae swam to the lighted area[22]; therefore, the larvae could be separated from their shells and then healthy larvae were cultivated and used to test the extracts of MP.

***Toxicity Test by the BSLT***

A brine shrimp (*A. salina Leach*) lethality assay is commonly used to check cytotoxic effects of bioactive chemicals. Shrimp eggs (0.25 g) were weighed on an analytical balance, and then grown in 300 mL of artificial seawater for 1-3 days. After 1-3 days, the eggs hatched, and became larvae (nauplii), and we selected healthy larvae for subsequent experiments. Concentrations of 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL of extracts of MP stems in artificial sea-water were prepared and placed in test tubes containing 10 nauplii, Nauplii were

evaluated, and dead ones were counted after 24 h. A dead larva indicated by a shrimp larva which did not show activity for a few seconds of observation[22].

***Pre-Culture of hOSCC Cells in T75 Flask***

*Human oral squamous cell carcinoma* (OM-1) cells stored in the vial preserved in a deep freezer (at -80 °C). Cells were thawed in sterile DIW at 37 ºC and moved to Falcon tubes with 5 mL of cell medium. The Falcon tubes were centrifuged at 500 rpm for 5 min. Cell medium consisted of high-glucose Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) and fetal bovine serum (FBS; Sigma-Aldrich) in a 90:10 ratio. The supernatant was removed and 5 mL of new medium was carefully added to each Falcon tube. The pellet (containing hOSCC cells) was gently resuspended in the medium and seeded into a flask. The flask was incubated for 2-3 days at 37 ºC and 5% $CO_2$ until a confluent monolayer of cells (80% density) was detected with an inverted microscope at 100x magnification (Nikon, Japan). Cells was detached using TrypLE™ Select Enzyme (Gibco, USA), harvested and counted with a cell counter (Scepter; Sigma-Aldrich), and the cells density was $1.3x10^6$/mL.

***Anticancer Activity Screening of MP Stem Extract against hOSCC Cells by An MTT Assay***

The microplate containing hOSCC cells was observed under an inverted microscope at a 100x magnification to ensure that cells were confluent. Each group consisted of three replicate wells (triplo). Treatment groups contained the MP stem ethanol extract or ethyl acetate extract at 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL. Two control groups were control medium that contained culture medium without cells and a control cell group that only contained cells and culture medium. Then, 25 µL of an extract was added to each well and incubated for 24 h at 37 ºC, and 5% $CO_2$. To each well was added 10 µL MTT (M2003; Sigma-Aldrich), and then the mixture was covered with aluminum foil and incubated for 4 h at 37 ºC. Lastly, 50 µL DMSO was added to each well and shaken vigorously, and then the absorbance level was measured by an enzyme-linked immunosorbent assay (ELISA) reader at a wavelength of 620 nm to obtain the $IC_{50}$ value[34].

***Measuring the Caspase-3 Level on hOSCC Cells Treated MP Stem Extract by ELISA***

The apoptotic activity of caspase-3 was measured with ELISA on hOSCC cells culture. The hOSCC cells were initially grown in 6-well plate with a seeding density of $4.10^5$ cells per well (2 ml volume). When the cells reached 85-90% confluence, cells were treated with 6 different concentrations of MP stem ethanol extract (0, 22.5, 45, 90, 360 ng/ml) and incubated for 0-, 2-, 24-, and 48-hours in 37 ºC, 5% $CO_2$. The complete DMEM growth medium without penicillin/streptomycin was used during the treatment. Every time points of treatment, a 1 ml of supernatant was collected for ELISA analysis. The sandwich ELISA kit (Bioenzy, Cat. BZ-08124084-EB) was used for a quantitative detection of Human Caspase-3. The collected supernatant was preliminary centrifuged to remove the debris and later transferred to 96-wells ELISA plate. The sample preparation was done by following the assay procedures described in the kit. Each sample was done in triplicate. As controls, untreated cells and serum-free medium were applied. Absorbance was determined at 450 nm using a microplate spectrophotometer (BioTek, Epoch, USA)[35].

***Statistical Analysis***

Data of larvae mortality were tabulated and assessed to obtain the $LC_{50}$ using a probit analysis[21]. However, the absorbance of the hOSCC culture was measured to obtain the $IC_{50}$ and caspase-3 level. All data were analyzed by GraphPad Prism 8th software. The concentration relationship between the ethanol and ethyl acetate extracts was assessed using a linear regression at a 95% confidence interval ($\alpha$=0.05).

## Results

### *Chemically Active Compounds of MP Stem Extract by GC-MS*

The components of the extracts were identified by comparing peak values of mass spectral fragmentation patterns to those of reference compound fragmentation patterns from the *Wiley Registry of Mass Spectral Data*. Several chemically bioactive compounds were identified in the ethanol extract of MP stems (Figure 1). The main components were 11.47% of 9, 12 octadecadienoic acid, 5.40% of hexadecanoic acid, methyl ester and pentadecanoic acid, 14-methyl which is palmitic acid (a group of saturated fatty acids). Meanwhile, other fatty acid groups with less than 5% concentrations included n-hexadecanoic acid, methyl stearate, heptadecanoic acid, 16-methyl, methyl ester, heptadecanoic acid, 14-methyl, methyl ester, and benzo (h) quinoline, 2, 4-dimethyl (Table I).

There were 15 peak values found by GC-MS in the ethyl acetate extract, but the concentrations were less than 5% (Figure 2). The main constituents were from the fatty acid family, with 4.62% oleic acid and octadec-9-enoic acid and, 2.08% 9.12-octadecadienoic acid (z, z). Using ethyl acetate as a solvent increased the extraction of chemically bioactive compounds from MP stem of not only palmitic acid, but also 1.21% of ethanol, 2-bromo, and other compounds with concentration of < 1%, such as 0.81% of 3'-chlorooxanilic acid N'(3-ethoxy-4-hydroxybenzylidene) hydrazide, 0.44% of 2-propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-3, 0.28% of cis-2,4,5-trimethoxy-.beta.-methyl-.beta.-nitrostyrene, 0.25% of 4-fluorobenzoic acid, 2-phenylethyl ester and 0.20% cyclopropaneoctanal, 2-octyl- (Table II).

### *Prediction of Biological Activities of Chemically Bioactive Compounds from MP Stem Extracts Using the Online PASS SERVER*

Some potentially bioactive compounds could be estimated through predictions using the online PASS SERVER. Based on results of chemically bioactive compound screening found using the GC-MS analysis, in general, compounds were members of the fatty acid group, such as *palmitic acid, linoleic acid, oleic acid,* and *stearic acid*. Chemically bioactive compounds with predicted potential were those that had a percentage of similarity to comparison database compounds of ethanol extract (Table I) and ethyl acetate extract (Table II) of MP stem that were greater than 90%. A compound's potential was predicted using a canonical smile obtained from the *PubChem online service* (Table III).

Predictions were based on results of online computing according to the PASS SERVER, and four fatty acid compounds had potential that could be used as a starting point of this study for anticancer agents. These compounds were palmitic acid, linoleic acid, enolic acid, and stearate. They were identified by examining Pa value in the activity of superoxide dismutase (SOD) inhibitors, apoptosis agonists, and caspase-3/caspase-8 stimulants. The data revealed that the bioactive compounds of hexadecanoic acid (palmitic acid) and pentadecanoic acid, 14-methyl, methyl ester had the highest Pa values against the activity of SOD inhibitors at Pa=0.914. The bioactive compounds with the highest apoptosis agonist activity were 9.12-octadecadienoic acid (z,z), methyl ester and cis-13-octadecadienoic acid, methyl ester (linoleic acid) with Pa=0.545. Furthermore, the bioactive compound with the highest activity as a caspase-3/caspase-8 stimulant was linoleic acid (Pa=0.617) (Table III).

The predicted result of bioactive compounds obtained Pa>0.7, indicating that the test compound's activity was the same as the drug's, and there were bioactive compounds with values of 0.5<Pa<0.7, indicating that the test compounds had lower biological activities than the drug. Table IV, shows predictions of hexadecanoic acid (palmitic acid) compounds with toxic activity against cancer cell cultures (lung carcinoma, metastatic melanoma, melanoma,

and stage 3 non-small cell lung cancer cells). In our results, predictions of toxic compounds from MP stem extract using CLC-Pred tools showed Pa>0.5.

***BSLT Results of MP Stem Extracts***

Brine shrimp lethality test (BSLT) is an acute toxicity test that applies a probit analysis to determine toxic concentration within 24 h. At each concentration, the death of shrimp larvae was measured, and a concentration-response relationship was determined using a regression analysis. The following equation was used to calculate the toxicity of the ethanol extract of MP stem:

$$y = 2.1669x + 1.5666 \quad (1)$$

Regression equation (1) achieved a value $R^2$=0.9824, so that the R calculated value was 0.9912. Based on the table value of R person, i.e., (R=0.666), which means the calculated R value was greater than the value from the R table. This indicated a significant correlation or relationship between the concentration and the percentage of shrimp larval death when the ethanol extract of MP stem was administered (Figure 3). There was a significant difference in the number of larval deaths between groups (p=0.027). In the probit analysis, the ethanol extract was found to be toxic at $LC_{50}$=38.41 µg/mL (Table V). The following regression equation was obtained to calculate the toxicity of the ethyl acetate extract of MP stem:

$$y = 1.8692x + 2.155 \quad (2)$$

In regression equation (2), $R^2$=0.9646 and R count =0.9821 were obtained. The R-value of the R person table was (R=0.666), indicating that R counts were greater than those of the R table. There was a significant correlation or relationship between the concentration of the ethyl acetate extract of MP stem and the percentage of shrimp larval death (Figure 3). There was a significant difference in the number of larval deaths between groups (p=0.010). The probit analysis revealed that ethyl acetate extract was toxic at $LC_{50}$=33.27 µg/mL (Table VI).

Whenever the results were compared to toxicity level criteria, the crude extract at $LC_{50}$<100 µg/mL was in the strong toxicity category, and crude extracts in the range of $LC_{50}$ values of 100<$LC_{50}$<500 µg/ml were classified as having moderate toxicity. The crude extracts with the range of $LC_{50}$ values for 500<$LC_{50}$<1,000 g/ml were considered to have low toxicity activity, while those with $LC_{50}$ values greater than 1,000 µg/mL were considered non-toxic.

***Cytotoxicity Assay of MP Stem Extracts to hOSCC Cells***

The cytotoxicity test results of MP stem sap deposited in a hOSCC (OM-1) cell culture revealed differences in the formazan formed in each group. Purplish crystal formation was observed in the reaction of MTT with the succinic dehydrogenase enzyme found in mitochondria (Figure 4). Formazan formed in each well was observed using an ELISA reader at a wavelength of 650 nm. The concentration inhibition of the ethanol extract of MP stem on hOSCC cells was 15.00 µg/mL ($IC_{50}$=15.00µg/mL). The inhibition of hOSCC cells was measured, and a concentration-response relationship was determined using a regression analysis. The following equation was used to calculate the concentration inhibition of the ethanol extract of MP stem against hOSCC culture:

$$y = -52.20x + 104.2 \quad (3)$$

Regression equation (3) obtained the value $R^2$=0.8769, so that the calculated value was R=0.9364. Based on the table value of R person, i.e., (R=0.344), this means that the calculated

R value was greater than the value from the R table. This indicates a significant correlation or relationship between the concentrations of the ethanol extract on inhibition of cancer cell (hOSCC) proliferation (Figure 5A). There was a significant difference in the inhibition of hOSCC cells between groups (p=0.000). However, the 50% inhibitory concentration ($IC_{50}$) of ethyl acetate extract was 10.61 µg/mL. The regression equation of the ethyl acetate extract was as follows:

$$y = -49.90x + 98.25 \quad (4)$$

Regression equation (4) obtained an $R^2$ value of 0.9321, so that the calculated R value was 0.9654. Based on the table value of R person I.e., (R=0.344), this means that the calculated R value was greater than the value from the R table. This indicates a significant correlation or relationship between the concentrations of the ethyl acetate extract on inhibition of cancer cell (hOSCC) proliferation (Figure 5B). There was a significant difference in the inhibition of hOSCC cells between groups (p=0.000)

***Apoptotic Activity of Caspase-3 on hOSCC Culture***

The time of incubation and concentration of MP stem ethanol extract had an influence on the concentration of caspase-3 in hOSCC culture. Whenever the MP stem ethanol extract was administered, the concentration of caspase-3 steadily increased after 2-, 24-, and 48-hours, as determined by ELISA. The Figure 6 revealed that administering an ethanol extract of MP stem at a dose of 180 ng/ml significantly increased caspase-3 levels compared to doses of 0, 22.5, 45, 90, 180, 360 ng/ml. However, after 2-, 24-, and 48-hours incubation, the quantity of caspase-3 was lower at the 0, 22.5, 45, 360 ng/ml than the 90 and 180 ng/ml. There was a significant difference for caspase-3 level in 2-hours (p=0.000), 24-hours (p=0.000), and 48-hours (p=0.000).

**Discussions**

Recent anticancer drug research focused on the genetic side with a fundamental goal in mind while avoiding damaging healthy cells[36]. Natural drugs can be an alternative to chemo- and radiotherapy[37]. The phytochemicals often exert their effects through modulating molecular pathways associated with the development and progression of cancer. Specific processes include boosting antioxidant status, inactivation of carcinogens, inhibition of cell growth, promotion of cell cycle arrest and apoptosis, and control of the immune system[38].

One of the mechanisms used in the development of anticancer drugs is the *p53* gene pathway. The *p53* gene is a tumor-suppressor gene that accumulates when DNA is damaged[39]. This gene serves as a controller of damaged cells dividing by inhibiting the cell cycle in the interphase phase/G1 and G2/M phase correction points[40]. Excessive cell stressors are captured by Toll-like receptors (TLRs), which then phosphorylate nuclear factor (NF)-κB and initiate the *p53* gene to perform apoptosis[41]. If stress cannot be eliminated by a cell, the *p53* gene mutates, and there is a failure of the process of apoptosis[35]. Failure of apoptosis allows damaged cells to continue to replicate and eventually form cancer[42]. Caspases are a type of proapoptotic protease that cleaves key sites for apoptosis execution. Caspase-3 is an important member of this family that causes apoptosis by inducing nuclear alterations. Many forms of cancer, such as cervical and breast cancer, have reduced caspase-3 levels, whereas elevated caspase-3 levels are a good predictive factor for malignancies[43].

Several chemically bioactive compounds were found in the ethanol extract, including hexadecanoic acid, methyl ester; n-hexadecanoic acid; pentadecanoic acid, 14-methyl, methyl ester; heptadecanoic acid, 16-methyl, methyl ester; and 9,12-octadecadienoic acid, which were palmitic acid and stearate acid group compounds (Table I). Palmitic acid, stearate acid, oleic

acid and linoleic acid group compounds were also found in the ethyl acetate extract, although at lower concentrations (Table II). In a previous study, members of the palmitic acid group contained a saturated long-chain fatty acid with a 16-carbon backbone that was shown to play a role in cancer suppression, particularly in colon cancer[44].

The concentration of hexadecanoic acid methyl ester had the highest percentage (5.40%) in the ethanol extract, while 9.12-octadecadienoic acid was 11.47% according to the results of the GC-MS analysis of the ethanol extract and ethyl acetate extract of MP stem. A previous study found that hexadecanoic acid compounds were a potential source of anti-inflammatory agents that selectively induced $G_2$/M arrest and apoptosis in MCF-7 cells via upregulation of *p53* and the Bax/Bcl-2 ratio[45,46], cytotoxicity against human leukemic cells, and inhibition phagocytic activity and nitric oxide production of certain cells[47], and could lower levels of TNF-α, prostaglandin E (PGE)-2, and interleukin (IL)-10 without affecting ATP levels[48,49]. Constrast, octadec-9-enoic acid and oleic acid had the potential to reduce inflammation, aid digestion, and promote probiotics and pro-omega 3[50]. It is possible that either of these identified compounds or other unknown compounds, or a synergistic effect of all these compounds combined contributed to the excellent anticancer and anti-inflammatory activity. The role of inflammation in cancer initiation and progression is well understood, and the underlying molecular mechanisms have been extensively studied. As a result, it has become feasible to target inflammatory pathways for cancer prevention and therapy in recent decades. DNA-repair proteins, caspases, lipid peroxidation, mutation, and NF-κB activation all contribute to a wide range of diseases, including cancer[51].

The PASS SERVER is commonly used to examine a compound's biological activity prior to chemical synthesis and biological testing. This method predicts the highest values of bioactive compounds based on a comparison of the Pa (probable active): Pi (probable inactive) ratio and the accuracy of the prediction was 95% based on a leave-one-out cross-validation (LOO CV) assessment[17,19]. This study found that hexadecanoic acid had a Pa=0.914 and Pi=0.003 values in the pathway via SOD inhibitors. In normal cells that experience oxidative stress, antioxidants in the body, such as SOD, dampen reactive oxygen species (ROS)[51]. High ROS and low SOD activities allow cell damage to continue, which can trigger DNA damage. Cells with damaged DNA can activate repair mechanisms through the apoptosis pathway by p53[52]. If cells with DNA damage are not immediately repaired, those cells may develop into abnormal cells (cancer). Nevertheless, antioxidant activity should be suppressed from another perspective. Some anticancer treatments, such as xenobiotics and radiation, kill tumor cells by releasing ROS. Cell with high amounts of such antioxidant enzymes are resistant to anticancer drugs[53]. The overexpression of SOD was associated with an increased incidence of tumor metastasis[54,55]. That was in accordance with this study in which potential compounds with the highest activity as agonists against apoptosis were linoleic acid (Pa=0.545) and a caspase-3/caspase-8 stimulant (Pa=0.617). Thus, the value of the Pa>Pi ratio indicated that it had the potential to be a good anticancer compound, because analysis of it using the CLC-Pred tools for in silico predictions of cell line cytotoxicity revealed that hexadecanoic acid exhibited toxicity to some cancer cell cultures such as melanomas. Therefore, MP stem extract might well inhibit the development of oral cancer (OSCC) due to its ability to induce apoptotis pathways.

Relying on the absorption computing system, distribution, metabolism, excretion, and toxicity (ADMET) screening, hexadecanoic acid or palmitic acid compounds could be well absorbed in the human intestinal tract (Pa=0.8417) because palmitic acid is a fatty acid that is highly fat-soluble, easily penetrates cell membranes, and was found in the subcellular milieu in mitochondria. This indicates that the ethanol and ethyl acetate extracts of banana stems could be orally administered.

*In vitro* testing should be used to validate the GC-MS data and computational predictions made with the PASS SERVER. They were calculated using the BSLT and Finney's *probit* analysis tool, and results showed that the $LC_{50}$ value of the ethanol extract was 38.41 µg/mL, and that of the ethyl acetate extract was 33.27 µg/mL, indicating that the ethyl acetate extract was more toxic than the ethanol extract. The $LC_{50}$ values for both extracts were less than 100 µg/m, and thus may be classified as highly toxic.

The use of ethanol and ethyl acetate extracts of MP stem in the culture method suppressed the growth of oral cancer (hOSCC) cells. The concentration of ethyl acetate extract that inhibited the proliferation of oral cells was lower than that of the ethanol extract. This was consistent with the wide range of chemicals discovered using the GC-MS technique on the ethyl acetate extract. As a result, when utilizing the ethanol extract to prevent cancer cell proliferation, a larger concentration will be necessary than when using the ethyl acetate extract. In the ethyl acetate extract, we found 15 chemically active compounds, and we found seven in the ethanol extract. The active compound of octadec-9-enoic acid and oleic acid which had potential as pro-omega 3. This would contribute to be a good anticancer.

In this study, apoptotic activity was observed by measuring the concentration of caspase-3 in hOSCC cell cultures treated with ethanol extract. The screening results through the GC-MS method showed that the concentration of palmitic acid derivative compounds, especially hexadecanoic acid, was higher in ethanol extracts. Compounds of hexadecanoic acid are predicted to have anticancer activity in several types of cancer, one of which is through the stimulation of caspase-3 activity. An increase in the concentration of caspase-3 in the application of ethanol extract of MP stem showed an increase in the apoptotic activity of cancer cells. The content of hexadecanoic acid can inhibit the growth of oral cavity cancer cells (hOSCC) according to the dose. There is a dose relationship with the administration of ethanol extract of MP stem at doses of $0.5IC_{50}$, $IC_{50}$, $2IC_{50}$, and $4IC_{50}$ to increase apoptotic activity by measuring caspase-3. However, there was a decrease in apoptotic activity with a hefty dose of $8IC_{50}$ (360mg/ml). A high-dose administration of MP stem ethanol extract in hOSCC cell cultures could inhibit apoptotic activity, which was indicated by low concentrations of caspase-3. As a result, the dose- and time-dependence of drug-induced apoptosis should be thoroughly assessed for both experimental and conventional anticancer agents.

Although *Musa paradisiaca var. sapientum (L) Kunz* extract has been used to treat various forms of cancer, its effect as an oral anticancer agent is still unknown. One of the limitations is that in silico studies (target and receptor) were not included in the computational stage of oral cavity anticancer screening, which was the focus of this current study. Caspases have a vital role in regulating apoptosis (programmed cell death) (apoptosis). Caspase-3 is a commonly activated death protease that cleaves numerous essential cellular proteins. However, this study can serve as a springboard for further development of an oral anticancer drug.

## Conclusions

We concluded that the chemically active compounds such as hexadecanoic acid obtained from the *Musa paradisiaca var. sapientum (L) Kunz* (MP) stem could be developed as candidates for new anticancer drugs. Hexadecanoic acid is palmitic acid derivates of MP stem extract has apoptotic activity by inhibiting cancer cell growth of hOSCC culture through caspase-3 stimulants.

## Acknowledgments

We'd like to express our gratitude to Universitas Airlangga for supporting research laboratories in conducting this study.

**Funding Statement**

The Ministry of Research and Technology /National Agency for Research and Innovation of Republic Indonesia on Project Number: 308/UN3.15/PT/2021.

**Authors' Contributions**

Conceptualization, H.S.B. and Y.K.S.; methodology, H.S.B. and Y.K.S.; investigation and data curation, N.M.U. and M.A.S.; data analysis, R.R.; resources, H.S.B.; writing-original draft preparation, H.S.B. and S.A.; writing-review and editing, supervision, H.S.B. Y.K.S. and C.Z.W. All authors contributed to the final version and approved the manuscript.

**Competing Interests**

We have no competing interests.

**Ethical Statement**

Ethics approval was not required for this study.

**Data Availability Statement**

The data used to support the finding of this study are included within the article.

**References**

1. Saraswat N, Pillay R, Everett B, George A. Knowledge, attitudes and practices of South Asian immigrants in developed countries regarding oral cancer: An integrative review. BMC Cancer 2020; 20: 477-493.
2. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The prevalence of squamous cell carcinoma in different sites of oral cavity at our rural health care centre in Loni, Maharashtra – a retrospective 10-year study. Contemp Oncol (Pozn) 2017; 2: 178-183.
3. Bugshan A, Farooq I. Oral squamous cell carcinoma: Metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res 2020; 9: 229.
4. Miranda-Filho A, Bray F. Global patterns and trends in cancers of the lip, tongue and mouth. Oral Oncol 2020; 102: 104551.
5. Ye L, Jiang Y, Liu W, Tao H. Correlation between periodontal disease and oral cancer risk: A meta-Analysis. J. Cancer Res Ther 2016; 12: 237-240.
6. Chen YH, Zou XN, Zheng TZ, Zhou Q, Qiu H, Chen YL, He M, Du J, Lei HK, Zhao P. High spicy food intake and risk of cancer: A meta-analysis of case–control studies. Chin Med J 2017; 130: 2241–2250.
7. Farah CS, Kujan O, Prime S, Zain RB. Oral mucosal malignancies chapter. Contemporary Oral Medicine. Springer Cham, 2019.
8. Zhang HH, Zhang Y, Cheng YN. Metformin in combination with curcumin inhibits the growth, metastasis, and angiogenesis of hepatocellular carcinoma in vitro and in vivo. Mol Carcinog 2018; 57: 44-56.
9. Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. Cell Death Dis 2019; 10: 177-189.
10. Li W, Jiang Z, Xiao X. Curcumin inhibits superoxide dismutase-induced epithelial-to-mesenchymal transition via the PI3K/Akt/NF-κB pathway in pancreatic cancer cells. Int J Oncol 2018; 52: 1593-1602.
11. Güçlü H, Doganlar ZB, Gürlü VP, Özal A, Dogan A, Turhan MA, Doganlar O. Effects of cisplatin-5-fluorouracil combination therapy on oxidative stress, DNA damage, mitochondrial apoptosis, and death receptor signalling in retinal pigment epithelium cells. Cutan Ocul Toxicol 2018; 37: 291-304.

12. Celentano A, McCullough M, Cirillo N. Glucocorticoids reduce chemotherapeutic effectiveness on OSCC cells via glucose-dependent mechanisms. J Cell Physiol 2019; 234: 2013-2020.
13. Kaae JK, Stenfeldt L, Eriksen JG. Xerostomia after radiotherapy for oral and oropharyngeal cancer: Increasing salivary flow with tasteless sugar-free chewing gum. Front Oncol 2016; 6: 111-116.
14. Al-Rubaye AF, Hameed IH, Kadhim M. A review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. Int J Toxicol Pharmacol Res 2017; 9: 948-953.
15. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: A review. Futur J Pharm Sci 2020; 6: 67.
16. Zhang A, Sun H, Wang X. Mass spectrometry-driven drug discovery for development of herbal medicine. Mass Spectrom Rev 2018; 37: 307-320.
17. Rudik A, Dmitriev A, Lagunin A, Filimonov D, Poroikov V. Metapass: A web application for analyzing the biological activity spectrum of organic compounds taking into account their biotransformation. Mol Inf 2021; 40: 103-110.
18. Chinnasamy P, Arumugam R. In silico prediction of anticarcinogenic bioactivities of traditional anti-inflammatory plants used by tribal healers in sathyamangalam wildlife Sanctuary, India. Egypt J Basic Appl Sci 2018; 5: 265-279.
19. Filimonov DA, Rudik AV, Dmitriev AV, Poroikov VV. Computer-aided estimation of biological activity profiles of drug-like compounds taking into account their metabolism in human body. Int J Mol Sci 2020; 21: 7492.
20. Lagunin AA, Dubovskaja VI, Rudik AV, Pogodin PV, Druzhilovskiy DS, Gloriozova TA, Filimonov DA, Sastry NG, Poroikov VV. CLC-Pred: A freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. PLoS ONE 2018; 13: e0191838.
21. Finney D. Probit analysis. J Pharm Sci 1971; 60: 1432.
22. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982; 45: 31-34.
23. Matata DZ, Ngassapa OD, Machumi F, Moshi MJ. Screening of plants used as traditional anticancer remedies in mkuranga and same districts, tanzania, using brine shrimp toxicity bioassay. Evid Based Complement Alternat Med 2018; 2018: 3034612.
24. Du L, Ma S, Wen X, Chai J, Zhou D. Oral squamous cell carcinoma cells are resistant to doxorubicin through upregulation of miR-221. Mol Med Rep 2017; 16: 2659-2667.
25. Chaudhary S, Chandrashekar KS, Pai KS, Setty MM, Devkar RA, Reddy ND, Shoja MH. Evaluation of antioxidant and anticancer activity of extract and fractions of Nardostachys jatamansi DC in breast carcinoma. BMC Complement Altern Med 2015; 15: 50.
26. Jyothirmayi N, Rao M. Banana medicinal uses. J Med Sci Tech 2015; 4: 152-160.
27. Sudirga S. Utilization of plants as traditional medicines in Trunyan village, Kintamani district, Bangli regency. J Bumi Lestari Udayana 2000; 12: 7-18.
28. Budi HS, Astuti ER. The MMP-2, MMP-9 expression and collagen density of the ambonese banana stem sap administration on wound healing. J Int Dent Med Res 2019; 12: 492-497.
29. Budi HS, Soesilowati P, Imanina Z. Gambaran histopatologi penyembuhkan luka pencabutan gigi pada makrofag dan neovaskular dengan pemberian getah batang pisang ambon. Maj Ked Gigi Indones 2017; 3: 121-127.
30. Deng W, Peng W, Wang T, Chen J, Zhu S. Overexpression of MMPs functions as a prognostic biomarker for oral cancer patients: A systematic review and meta-analysis. Oral Health Prev Dent 2019; 17: 505-514.

31. Huang H. Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: Recent advances. Sensors 2018; 18: 3249.
32. Azmin SNHM, Manan ZA, Alwi SRW, Chua LS, Mustaffa A, Yunus NA. Herbal processing and extraction technologies. Sep Purif Rev 2016; 45: 305-320.
33. Budi HS, Juliastuti WS, Ariani W. MTT-based cytotoxic evaluation of ambonese banana stem sap (Musa paradisiaca var. Sapientum (L.) Kuntze) on fibroblast cells. Period Tche Quim 2020; 17: 558-564.
34. Freshney RI. Culture of animal cells: A manual of basic technique and specialized applications. Wiley-Blackwell, 2016.
35. Ju SM, Bae JS, Jeon BH. AMP-activated protein kinase contributes to ROS-mediated p53 activation in cisplatin-induced nephrotoxicity. Eur Rev Med Pharmacol Sci 2021; 25(21): 6691-6700.
36. Maeda H, Khatami M. Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. Clin Transl Med 2018; 7: 11-30.
37. Zhang QY, Wang FX, Jia KK, Kong LD. Natural product interventions for chemotherapy and radiotherapy-induced side effects. Front Pharmacol 2018; 9: 1253.
38. Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. Front Pharmacol. 2020;11:175.
39. Zhu G, Pan C, Bei JX, Li B, Liang C, Xu Y, Fu X. Mutant p53 in cancer progression and targeted therapies. Front Oncol. 2020;10:595187.
40. Liu K, Zheng M, Lu R, Du J, Zhao Q, Li Z, Li Y, Zhang S. The role of CDC25C in cell cycle regulation and clinical cancer therapy: a systematic review. Cancer Cell Int. 2020;20:213.
41. Zhang DX, Ma DY, Yao ZQ, Fu CY, Shi YX, Wang QL, Tang QQ. ERK1/2/p53 and NF-κB dependent-PUMA activation involves in doxorubicin-induced cardiomyocyte apoptosis. Eur Rev Med Pharmacol Sci. 2016;20(11):2435-42.
42. Zheng TL, Cen K. MiR-92a inhibits proliferation and promotes apoptosis of OSCC cells through Wnt/β-catenin signaling pathway. Eur Rev Med Pharmacol Sci. 2020;24(9):4803-4809.
43. Boice A, Bouchier-Hayes L. Targeting apoptotic caspases in cancer. Biochim Biophys Acta Mol Cell Res. 2020;1867(6):118688.
44. Arun KB, Madhavan A, Reshmitha TR, Thomas S, Nisha P. Musa paradisiaca inflorescence induces human colon cancer cell death by modulating cascades of transcriptional events. Food Funct 2018; 9: 511-524.
45. Ghate NB, Das A, Chaudhuri D, Panja S, Mandal N. Sundew plant, a potential source of anti-inflammatory agents, selectively induces G2/M arrest and apoptosis in MCF-7 cells through upregulation of p53 and Bax/Bcl-2 ratio. Cell Death Discov 2016; 2: 15062.
46. Achakzai JK, Anwar Panezai M, Kakar MA, Kakar AM, Kakar S, Khan J, Khan NY, Khilji I, Tareen AK. In vitro anticancer MCF-7, anti-inflammatory, and brine shrimp loethality assay (BSLA) and GC-MS analysis of whole plant butanol fraction of rheum ribes (WBFRR). BioMed Res Int 2019; 2019: 3264846.
47. Mellado M, Soto M, Madrid A, Montenegro I, Jara-Gutiérrez C, Villena J, Werner E, Godoy P, Aguilar LF. In vitro antioxidant and antiproliferative effect of the extracts of Ephedra chilensis K Presl aerial parts. BMC Complement Altern Med 2019; 19: 53.
48. Mericli F, Becer E, Kabadayı H, Hanoglu A, Yigit Hanoglu D, Ozkum Yavuz D, Ozek T, Vatansever S. Fatty acid composition and anticancer activity in colon carcinoma cell lines of Prunus dulcis seed oil. Pharm Biol 2017; 55: 1239-1248.

49. Perry BD, Rahnert JA, Xie Y, Zheng B, Woodworth-Hobbs ME, Price SR. Palmitate-induced ER stress and inhibition of protein synthesis in cultured myotubes does not require Toll-like receptor 4. PLoS One. 2018;13(1):e0191313.
50. Farag MA, Gad MZ. Omega-9 fatty acids: potential roles in inflammation and cancer management. J Genet Eng Biotechnol. 2022;20(1):48.
51. Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biol 2019; 25: 101084.
52. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: Harms and benefits for human health. Oxid Med Cell Longev 2017; 2017: 8416763.
53. Ma X, Deng D, Chen W. Inhibitors and activators of SOD, GSH-Px, and CAT: Chapter. Enzyme inhibitors and activators. Intechopen, 2017.
54. Griess B, Tom E, Domann F, Teoh-Fitzgerald M. Extracellular superoxide dismutase and its role in cancer. Free Radic Biol Med 2017; 112: 464-479.
55. Che M, Wang R, Li X, Wang HY, Zheng XFS. Expanding roles of superoxide dismutases in cell regulation and cancer. Drug Discov Today 2016; 21; 143-149.

## Table captions

**Table I.** GC-MS analysis of chemically active compounds in *Musa paradisiaca var. sapientum (L) Kunz* ethanol extract

| Peak | Retention time | Peak area (%) | Chemically active compounds | Qual |
|---|---|---|---|---|
| 1 | 15.815 | 5.40 | Hexadecanoic acid, methyl ester | 98 |
| | | | Pentadecanoic acid, 14-methyl-, methyl ester | 97 |
| 2 | 15.996 | 3.85 | n- Hexadecanoic acid | 95 |
| 3 | 16.930 | 2.29 | Methyl stearate | 91 |
| | | | Heptadecanoic acid, 16-methyl-, methyl ester | 91 |
| | | | Heptadecanoic acid, 14-methyl-, methyl ester | 90 |
| 4 | 17.024 | 11.47 | 9,12-Octadecadienoic acid | 46 |
| 5 | 17.101 | 0.96 | Benzo (h) quinoline, 2,4-dimethyl | 38 |

**Table II.** GC-MS analysis of chemically active compounds in *Musa paradisiaca var. sapientum (L) Kunz* ethyl acetate extract

| Peak | Retention time | Peak area (%) | Chemically active compounds | Qual |
|---|---|---|---|---|
| 1 | 14.924 | 0.28 | Cis-2,4,5-Trimethoxy-.beta.-methyl-.beta.-nitrostyrene | 38 |
| 2 | 15.055 | 0.14 | Triamterene | 41 |
| 3 | 15.224 | 0.25 | 4-Fluorobenzoic acid, 2-phenylethyl ester | 27 |
| 4 | 15.815 | 1.73 | Pentadecanoic acid, 14-methyl-, methyl ester | 99 |
| | | | Hexadecanoic acid, methyl ester | |
| 5 | 15.997 | 1.33 | n- Hexadecanoic acid | 99 |
| 6 | 16.768 | 0.39 | Methyl 10-trans, 12-cis-octadecadienoate | 99 |
| | | | 9,12-Octadecadienoic acid (z,z)-, methyl ester | 99 |
| | | | 9,15-Octadecadienoic acid (z,z)-, methyl ester | 99 |
| 7 | 16.796 | 0.68 | 9-Octadecadienoic acid (z)-, methyl ester | 99 |
| | | | Cis-13-Octadecadienoic acid, methyl ester | 99 |
| 8 | 16.931 | 0.98 | Methyl stearate | 98 |
| 9 | 17.021 | 4.62 | Oleic acid | 96 |
| | | | Octadec-9-enoic acid | 96 |
| 10 | 17.145 | 2.08 | 9,12-Octadecadienoic acid (z,z)-, Oleic acid | 95 |
| 11 | 17.788 | 0.20 | Cyclopropaneoctanal, 2-octyl- | 90 |
| 12 | 18.850 | 0.81 | 3'-Chlorooxanilic acid N'(3-ethoxy-4-hydroxybenzylidene) hydrazide | 42 |
| 13 | 19.065 | 1.21 | Ethanol, 2-bromo- | 35 |
| 14 | 19.432 | 0.44 | 2-propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-3 | 64 |
| 15 | 19.993 | 0.19 | Oleic acid | 47 |

**Table III.** *Canonical smile* of fatty acid compounds group has anticancer potential in *Musa paradisiaca var. sapientum (L) Kunz*

| No | Chemically active compounds | Synonym | Canonical SMILE | Pa value | Activity |
|---|---|---|---|---|---|
| 1 | Hexadec-anoic acid, methyl ester | Palmitic acid methyl ester<br>Methyl palmitate | CCCCCCCCCCCCCCCC(=O)OC | 0.738 | Superoxide dismutase inhibitor |
| | | | | 0.473 | Apoptosis agonist |
| | | | | 0.609 | Caspase 3 and 8 stimulant |
| 2 | Pentade-canoic acid, 14-methyl-, methyl ester | Pentadecanoic acid<br>Pentadecylic acid<br>1002-84-2<br>n-Pentadecanoic acid<br>Pentadecyclic acid | CCCCCCCCCCCCCCC(=O)O | 0.914 | Superoxide dismutase inhibitor |
| | | | | 0.342 | Apoptosis agonist |
| | | | | 0.562 | Caspase 3 and 8 stimulant |
| 3 | n- Hexadec-anoic acid | Palmitic acid<br>Hexadecanoic acid<br>57-10-3<br>Cetylic acid<br>Palmitate | CCCCCCCCCCCCCCCC(=O)O | 0.914 | Superoxide dismutase inhibitor |
| | | | | 0.342 | Apoptosis agonist |
| | | | | 0.562 | Caspase 3 and 8 stimulant |
| 4 | Methyl stearate | Methyl octadecanoate<br>112-61-8<br>Octadecanoic acid, methyl ester<br>Stearic acid methyl ester | CCCCCCCCCCCCCCCCCC(=O)OC | 0.738 | Superoxide dismutase inhibitor |
| | | | | 0.336 | Apoptosis agonist |
| | | | | 0.609 | Caspase 3 and 8 stimulant |
| 5 | Heptade-canoic acid, 16-methyl-, methyl ester | Methyl heptadecanoate<br>1731-92-6<br>Methyl margarate<br>Heptadecanoic acid, methyl ester<br>Margaric acid methyl ester | CCCCCCCCCCCCCCCCC(=O)OC | 0.738 | Superoxide dismutase inhibitor |
| | | | | 0.473 | Apoptosis agonist |
| | | | | 0.609 | Caspase 3 and 8 stimulant |
| 6 | 9,12-Octadec-adienoic acid (z,z)-, methyl ester<br>Cis-13-Octadecadieno ic acid, methyl ester | Linoleic acid<br>60-33-3<br>Linolic acid<br>Telfairic acid<br>cis,cis-Linoleic acid | CCCCCC=CCC=CCCCCCCCC(=O)O | 0.814 | Superoxide dismutase inhibitor |
| | | | | 0.545 | Apoptosis agonist |
| | | | | 0.617 | Caspase 3 and 8 stimulant |
| 7 | Oleic acid<br>Octadec-9-enoic acid | oleic acid<br>112-80-1<br>cis-9-Octadecenoic acid<br>(Z)-Octadec-9-enoic acid<br>cis-Oleic acid | CCCCCCCCC=CCCCCCCCC(=O)O | 0.851 | Superoxide dismutase inhibitor |
| | | | | 0.499 | Apoptosis agonist |
| | | | | 0.592 | Caspase 3 and 8 stimulant |
| 8 | | | CCCCCCCCC1CC1CCCCCCCC=O | 0.653 | Superoxide dismutase inhibitor |

| | | | | | |
|---|---|---|---|---|---|
| | Cyclopr-opaneoctanal, 2-octyl- | 2-Octylcyclopropaneoctanal<br>8-(2-Octylcyclopropyl)octanal<br>2-octyl-cyclopropaneoctanal | | 0.386 | Apoptosis agonist |
| | | | | 0.411 | Caspase 3 and 8 stimulant |

**Table IV.** Toxicity of *hexadecanoic acid* on cell cancer culture used the *CLC- Pred tools*

| Pa | Pi | Cell-line | Cell-line full name | Tissue | Tumor type |
|---|---|---|---|---|---|
| 0.558 | 0.017 | DMS-114 | Lung carcinoma | Lung | Carcinoma |
| 0.545 | 0.017 | SK-MEL-1 | Metastatic melanoma | Skin | Melanoma |
| 0.521 | 0.009 | A2058 | Melanoma | Skin | Melanoma |
| 0.537 | 0.033 | NCI-H838 | Non-small cell lung cancer (stage 3) | Lung | Carcinoma |

**Table V.** *Probit* analysis of Brine Shrimp Lethality Test on *Musa paradisiaca var. sapientum (L) Kunz* ethanol extract

| Concentration | Log concentration | Life | Mortality | Probit | $LC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 10 | 1 | 9 | 10% | 3.72 | 38.41276 |
| 20 | 1.30103 | 7 | 30% | 4.48 | |
| 30 | 1.477121 | 6 | 40% | 4.75 | |
| 40 | 1.60206 | 5 | 50% | 5.00 | |
| 50 | 1.69897 | 4 | 60% | 5.25 | |
| 60 | 1.778151 | 4 | 60% | 5.25 | |
| 70 | 1.845098 | 3 | 70% | 5.52 | |
| 80 | 1.90309 | 2 | 80% | 5.84 | |
| 90 | 1.954243 | 2 | 80% | 5.84 | |

**Table VI.** *Probit* analysis of *Musa paradisiaca var. sapientum (L) Kunz* on ethyl acetate extract

| Concentration | Log concentration | Life | Mortality | Probit | $LC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 10 | 1 | 8 | 20% | 4.16 | 33.26914 |
| 20 | 1.30103 | 7 | 30% | 4.48 | |
| 30 | 1.477121 | 6 | 40% | 4.75 | |
| 40 | 1.60206 | 4 | 60% | 5.25 | |
| 50 | 1.69897 | 4 | 60% | 5.25 | |
| 60 | 1.778151 | 3 | 70% | 5.52 | |
| 70 | 1.845098 | 3 | 70% | 5.52 | |
| 80 | 1.90309 | 2 | 80% | 5.84 | |
| 90 | 1.954243 | 2 | 80% | 5.84 | |

**Figure captions**

**Figure 1.** GC-MS chromatogram of MP stem ethanol extract.
**Figure 2.** GC-MS chromatogram MP stem ethyl acetate extract.
**Figure 3.** Correlation between extract concentration and Brine Shrimp Lethality Test. The orange line represented MP stem ethanol extract, and the blue line represented MP stem ethyl acetate extract.
**Figure 4.** Cytotoxicity assay of hOSCC culture by MTT method. A. Seeding cell, B. Harvesting cell, and C. Formazan formation.
**Figure 5.** Inhibition concentration of MP stem extract to hOSCC culture. A. $IC_{50}$ of ethanol extract and B. $IC_{50}$ of ethyl acetate extract
**Figure 6.** Caspase-3 level on hOSCC culture treated MP stem ethanol extract. A. 96 well plate scheme, B. Concentration of caspase-3 in different doses and incubation time. Conc.: concentration.

# Your article #27067 is Accepted

**Publishing Manager** <info@publishingmanager.org>
Reply-To: "info@publishingmanager.org" <info@publishingmanager.org>
To: hendrik-s-b@fkg.unair.ac.id

Fri, Sep 2, 2022 at 5:55 PM

Dear Doctor,

your paper "Palmitic acid of Musa Paradisiaca induce apoptosis through caspase-3 in human oral squamous cell carcinoma" has been accepted for publication in European Review for Medical and Pharmacological Sciences.

**Communication:** Reference Number: Art. 12057-PM 27067..

The reference number of the article is **#27067**.

The price of publication is **1600€**. For payments from Italy the final price does not include VAT (+22%).

The details of payment are in this page.

You have 30 working days to complete payment.

Once finalized the payment, you will receive the galley proof.

Please note that requests on publication fee waivers have to be made before initial submissions and not after acceptance. Authors coming from the countries included in the "United Nations list of Least Developed Countries" are entitled to ask for a discount.

Best regards,
Journal Editorial Team

# #1.12057

**Verduci Editore** <submission@europeanreview.org>
To: hendrik setia budi <hendrik-s-b@fkg.unair.ac.id>

Tue, Sep 20, 2022 at 2:14 PM

Dear Dr,

the galley proof of article #1.12057 is attached.

Please, send your approval.

Best regards,

Journal Editorial Team

Verduci Publisher
Via Gregorio VII, 186
00165 Roma
E-mail: submission@europeanreview.org
www.europeanreview.org
www.verduci.it

**Art. 12057-PM 27067-Hendrik Setia Budi-SUPERFAST.pdf**
1646K

# #1.12057 URGENT

**Verduci Editore** <submission@europeanreview.org>
To: ykshen@tmu.edu.tw, hendrik setia budi <hendrik-s-b@fkg.unair.ac.id>

Fri, Oct 7, 2022 at 5:23 PM

Dear Dr,

the final galley proof of article #1.12057 is enclosed.

Please, send your approval.

**Before publication, we need you to**
**• send us Figures 1 and 2 at a better resolution**
**• send us Figure 3 with the point instead of the comma for decimal numbers**
**• add magnifications for Figure 5**
**• send us Figure 6 with the point instead of the comma for decimal numbers**

**Note that we cannot add Prof in the Corresponding Author section as we always put MD, Ph.D. The same goes for MDS. If it's a Master of Science we generally put MSc. Please, let us know.**

Please, use the attached file to make corrections.

The article will be published in the 1st issue of October.

**Note: Please, check galley proof within 3 days.**

**After this time, corrections will not be done.**

**Please, check the galley proof carefully.**

Thank you in advance for your cooperation.

Best regards,

Journal Editorial Team

Verduci Publisher
Via Gregorio VII, 186
00165 Roma
E-mail: submission@europeanreview.org
www.europeanreview.org
www.verduci.it

**Art. 12057-PM 27067-Hendrik Setia Budi.pdf** 1623K

# Your article #27067 has been published online

**Publishing Manager** <info@publishingmanager.org>
Reply-To: "info@publishingmanager.org" <info@publishingmanager.org>
To: hendrik-s-b@fkg.unair.ac.id

Wed, Mar 15, 2023 at 11:26 PM

Dear Doctor,

thanks for choosing European Review for Medical and Pharmacological Sciences to publish your study.

Your article has been published online and is available on our website.

At this link, you will find an experience survey to express your personal assessment of the publishing experience.

The present questionnaire is aimed at providing insights into our publishing process to satisfy our authors and to perfect our peer-review process in the future.

All constructive feedback whether positive or negative is very much appreciated.

Thanks for your cooperation.

Best regards,
Journal Editorial Team