Chapter

Antioxidant Activity of Areca Nut to Human Health: Effect on Oral Cancer Cell Lines and Immunomodulatory Activity

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Abstract

Many herbs have been discovered to be potential sources of the antitumor and immunomodulatory drug. Areca nut (*Areca catechu* L.) has a high content of phenols and flavonoids and is highly related to antioxidant activity. Areca nut is a traditional herbal medicine that is popular around Indonesia, India, Thailand, and Taiwan. However, data on its effect on human health showed various results. This chapter's aim to review the phytochemical and polyphenolic content, the molecular structure of bioactive compounds, the side effect of the crude extract, the role of catechin in cancer mechanism, the antioxidant activity, the cytotoxicity, and immunomodulatory activity of the areca nut. Areca nuts from Aceh province in Indonesia, contain flavonoids, phenolics, catechin, quercetin, and a small percentage of tannins which contribute to antioxidant activity. The areca nut has anticancer potential activity so it can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy-induced toxicity. Areca nuts also show immunomodulatory activity which can increase the body's immune system.

Keywords: Areca nut, phytochemical content, anticancer, immunomodulatory

1. Introduction

One of the plants with the potential to be developed as herbal medicine is the *pinang* plant (*Areca catechu* Linn; *areca*, *Palmaceae*). The term *Areca catechu* refers to the largest amount of the phytochemical compounds in areca nut that is arecoline and catechin. Areca nut is a traditional herbal medicine that is popular around Indonesia, India, Thailand, and Taiwan. This plant spreads widely in South East Asia, South Africa, and the Pacific Ocean Islands. It is usually used in betel chewing common among the Indians and Malays as a breath freshener, digestive aid, anthelmintic, aphrodisiac, and to maintain stamina [1]. Several kinds of research studied the functions of the areca plant showed that the stem can be used as antibacterial and antifungal [2]. Epidemiologic study and laboratory research found a large number of anticancer compounds obtained from the nuts. Some of them contained flavonoid, polyphenol, carotenoid, selenium, vitamin C, and E which showed chemotherapy and preventive activity. An antioxidant is one of the solutions to

minimize oxidative stress levels caused by cancer treatment. The antioxidant is a compound that is capable to cleanse, dispose, and repel free radical or reactive oxygen species forming in the body [3]. The previous study showed that methanol and water extract of areca nut contains tannin and high total phenolic [4–6]. This compound has stronger free radical scavenging activity than ascorbic acid [7].

2. Phytochemical content of areca nut

Determination of areca plant as follows:

Kingdom	*: Plantae*
Division	*: Magnoliophyta*
Subdivision	*: Angiospermae*
Class	*: Liliopsida*
Nation	*: Arecales*
Family	*: Arecacea/palmae*
Subfamily	*: Arecoideae*
Genus	*: Areca*
Species	*: Areca catechu* Linn.

Characteristics of areca nut	
Stems	Slender, grow upright, reach 10 to 30 m high, 15 to 20 cm in diameter, and unbranched [8]. The new stem formation occurs after two years (**Figure 1**).
Leaves	The pinnate compound leaves grow together at the tip of the stem, almost like coconut trees. Leaf midrib tubular has 80 cm long and short petiole. It has 1–1.8 cm long, 5 cm wide, with torn and jagged tips.
Flowers	Flower cobs with long, easy-to-fall spathes appear under the leaves. They appear at the beginning and the end of the rainy season.
Nuts	• Oval elongated has 3.5 to 7 cm long, fibrous mesocarp and a thin woody endocarp enveloping one nut. • The color of the ripe nut is red-orange (**Figure 2**). • Sweet, fresh, and it gives a sense of addiction. • The nuts appear at the age of 5 to 8 years depending on the soil condition.
Roots	Fiber roots and very similar to the roots of the coconut plants.
Life span	25 to 30 years.
Grow locations	Areca plants can produce optimally when planted in locations with an altitude of 0–1,400 masl. The required rainfall to grow areca nut optimally is between 2,000–3,000 mm/year which is evenly distributed throughout the year or rainy days around 100–150 days. The desired temperature is 20 °C–32 °C, and the humidity is between 50–90%. The soil acidity which is good for plant growth around pH 4–8 [9].
Constituents	• **Polyphenols**: **Phenolics, flavonoids**, syringic acid, procyanidin (dimeric, trimeric, and tetrameric), tannins, isorhamnetin 3-O-rutinoside, **catechin**, jacareubin, ent-catechin, epicatechin, **quercetin**, luteolin, and chrysoeriol [4, 5]. • **Alkaloids**: Arecoline, arecaidine, guvacoline, and guvacine. Arecoline is colorless volatile resembling nicotine. • **Fatty acids**: Myristic acid, palmitic acid, oleic acid, linoleic acid, eicosanoic acid, arachidonic acid, docosanoic acid, tetracosanoic acid, hexacosanoic • Carbohydrate (19.13%), protein (10.22%), fats (12.84%), crude fibers (14.40%),

	Characteristics of areca nut
	• Minerals: Calcium, phosphorus, iron, vitamin B6, and C.
Actions	• Antioxidant, anti-aging, antihelminthic, antimicrobial, analgesic, and anti-inflammatory [2, 10–13]. • It can reduce the risk of dental caries. • Apoptosis and cell cycle arrest in carcinoma cell lines [14]. • Anti-inflammatory/Anti-melanogenesis • Hypolipidemic • Hypoglycemic activity • Antidepressant • A-glucosidase inhibitory • Antihypertensive • Immunomodulatory activity • Vascular relaxation • Anticonvulsant activity • Anti-allergic
Side effects	• Chewing habits can induce the risk of developing oral squamous cell carcinoma. • The arecolines can enhance Alzheimer's type of dementia. • Dental attrition, oral leukoplakia, areca staining, lichenoid lesions.

Areca nut contains several alkaloids that belong to the pyridine group, especially arecoline. Arecoline affects the oculomotor nerve which can cause mydriasis, mild

Figure 1.
Areca plants [8].

Figure 2.
The ripe areca nuts.

paralysis, and pupillary dilatation [15]. Several studies showed that arecoline can induce neurotoxicity through its action in oxidative stress and generating Reactive Oxygen Species (ROS), hepatotoxicity, testicular toxicity, oropharyngeal cancers, and oral submucous fibrosis [16, 17]. Besides alkaloids, ent-catechin, and jacareubin are the major compound in areca nut [7]. Catechin and its analogs are antioxidant, anti-allergic, anticancer, anti mutation, and anti-inflammatory. It can improve liver function and scavenge free radicals [18]. The beneficial effect of catechins is reported in the treatment of cancer, cardiovascular diseases, diabetes, neurodegenerative diseases, and liver diseases [19]. Apparently, catechins have function not only as a powerful antioxidant [20], preventing oxidative damage in healthy cells [21], but also as an antiangiogenic, antitumor agent [22], and a modulator of tumor cell response to chemotherapy [23]. It can induce apoptosis by increasing caspases [24] and promotes cell growth arrest by altering the expression of cell cycle regulatory proteins [25]. Jacareubin is reported to have an anti-inflammatory property and increases the activity of H^+, K^+-ATPase which is needed to gain the energy of the cells [26, 27].

The majority of phytochemical ingredients in areca nut extract are phenolic compounds such as flavonoids, tannins, and alkaloids [28]. It was established that a great quantity of tannins has been found in dark dry fruits such as tea. The areca nut also has a reddish-brown color. The levels of tannin found in areca nut have different contents in different areas or regions [29]. It could be caused by climate conditions and environmental factors where it grows. Climatic factors such as temperature, weather, and rainfall. The environmental factors such as soil type and fertility, the height of growing, and plant maintenance.

The presence of catechin and quercetin mostly were identified through High-Performance Liquid Chromatography (HPLC) analysis. These two compounds are well-known antioxidants and could have contributed to the observed antioxidant activity [30]. Previous studies have identified several phenolic compounds in the areca nuts including trimer procyanidin, dimer procyanidin (B1 dan B2), catechin, and isorhamnetin 3-O-rutinoside [31]. Many of these compounds have antioxidants activities [20, 32]. Catechin was proved to have strong antioxidant activity which could contribute towards the anti-cancer effects [33–36]. It has also been shown that polymerized catechin suppresses the activity of *Staphylococcus aureus* α-toxin and as an effective urease inhibitor in *Staphylococcus saprophyticus* strains. Although the level of quercetin is not much in areca nut extract, it also has been proved as an antioxidant. It possesses an anti-inflammatory potential that can be expressed in different cell types, both in animal and human models [37]. Quercetin is also able to inhibit the growth of cancer cells through induction of apoptosis and inhibitory proliferation in gastrointestinal, breast, esophagus, and ovary cancer [38].

3. Quantitative data of polyphenolic content in areca nut

Various studies have been conducted to calculate the content of polyphenolic areca nut from various regions around the world. The results also showed various results. This difference shows that the variation in polyphenolic content depends on the geographical conditions in which the plant grows so that it affects the quality and quantity of the phytochemical composition in areca nut. This variation is also influenced by the species and type of the areca nuts used such as freshness, maturity, and form of the nut drying process. One of the polyphenolic content derived from areca nut on the island of Sumatra in Indonesia is shown in **Table 1**. The total phenolic and flavonoid contents of areca nut are expressed as Tannin Acid Equivalents (TAE) and Catechin Equivalents (CE).

Methanolic extract	Compounds	Estimated amount
Ripe areca nut	Total phenolics	80.3 mg TAE/gr[a]
	Total flavonoids	238.5 mg CE/gr[b]
	Catechin	2.79 mg/gr[c]
	Quercetin	0.14 mg/gr[c]
	Tannin	0.007%[d]
Unripe areca nut	Total phenolics	56.6 mg TAE/gr[a]
	Total flavonoids	18.13 mg CE/gr[b]
	Catechin	—
	Quercetin	—
	Tannin	8.7%[d]

[a]*Folin–Ciocalteu method.*
[b]*Dowd method.*
[c]*HPLC.*
[d]*Titrimetri assay.*

Table 1.
Quantitative data of polyphenolic content in areca nut from Aceh, Indonesia.

4. Molecular structure of bioactive compounds in areca nut

4.1 Phenolic compounds

Phenolic compounds are the largest part of the phytochemicals in plants, especially fruit, seeds, and leaves. This collection of compounds provides many health benefits because they contain many antioxidants that can scavenge oxidative stress due to reactive oxygen species. Intake of fruit, vegetables, and whole grains that are rich in phenolics can lower the risk of cardiovascular disease, chronic inflammation, cancer, and neurodegenerative diseases. Polyphenolics contain an aromatic hydroxyl ring derived from L-phenylalanine. Phenolic compounds comprise one (phenolic acids) or more (polyphenols) aromatic rings with attached hydroxyl groups in their structures. Several types of polyphenols that are classified as phenolic acids are hydrolyzed tannins, lignans, stilbene, and flavonoids. The intact polyphenolic form is usually absorbed directly from the digestive tract.

Microorganisms can cause the degradation of polyphenolics into aglycones and aromatic acids. Polyphenolics are detected in all tissues, especially the digestive tract and oral mucosa. All types of polyphenolics are excreted in the urine and bile ducts. The bioavailability of polyphenolics is low, and the remaining at urinary excretion is 0.3% for anthocyanins and 43% for isoflavones. The absorption of phenolic compounds in food is determined by the chemical structure which influences the degree of glycosylation and acylation, basic structure, conjugation with other phenolic compounds, molecular size, polymerization, and solubility. The maximum concentration in plasma rarely exceeds 1 mM after ingestion of 10–100 mg of a single phenolic compound. Polyphenolics that are mostly absorbed by the body are isoflavones, gallic acid, flavanones, catechins, and quercetin glycosides, while proanthocyanidins, anthocyanins, and gallolylated catechins are less widely absorbed.

4.2 Flavonoid

The total flavonoids in ripe areca nuts were found to be the highest in levels. Flavonoids belong to a group of natural substances with variable phenolic structures

and are found in vegetables, tea, flowers, fruit, grains, vegetables, grains, and wine. This natural ingredient is known to have many health benefits. Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. These compounds possess a common phenylbenzopyrone structure (C6-C3-C6), and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols. Flavonoids are polyphenolic compounds that consist of several types based on their structure, namely flavonols (quercetin and kaempferol), flavones (luteolin and wogonin), flavanols (catechin, gallocatechin), isoflavones (genistein), flavanones, and flavanonols.

Flavonoids have an antibacterial, anti-inflammatory, antioxidant, allergy, antimutagenic, antiviral, antineoplastic, anti-thrombotic, vasodilatory, and anti-hepatotoxic activities. Flavonoids can cause cell cycle arrest, thereby inhibiting the activity of Cyclin-Dependent Kinases (Cdks), Phosphorylation Kinases (PKs), and signal transduction of cell proliferation. Flavonoids are also able to modulate Mitogen-Activated PKs (MAPKs). The Cdk enzyme is an enzyme that functions to control the activity of the phosphorylase enzyme which regulates every phase of the cell cycle, especially during DNA replication and chromosome formation. The anti-tumor properties of flavonoids can inhibit the release of prooxidant enzymes, modulate carcinogen metabolism, suppress protein tyrosine kinase activity, anti-proliferation, anti-metastasis, inhibit some drug resistance, are antioxidant and anti-angiogenesis, induce apoptosis and cell cycle retention. The chemical structures of flavonoids in areca nut can be seen in **Figure 3**.

4.3 Catechin

Catechins are flavonoids (flavanols) that are included in the polyphenol group, which have high concentrations in vegetables, fruits, and beverages. Catechins contain two aromatic rings and several hydroxyl groups. Catechins are divided into two groups, namely free catechins and esterified catechins. Catechin, gallocatechin, epicatechin, epigallocatechin are non-esterified catechins, whereas epigallocatechin gallate, epicatechin gallate, gallocatechin gallate are classified as esterified. The chemical structure of catechins can be seen in **Figure 4**.

Several *in vitro* studies have proven the role of catechins as protection for several diseases such as degenerative, heart disease, and bacterial infection. The catechins in green tea are able to inhibit carcinogenesis of the skin, lungs, esophagus, stomach, liver, small intestine, colon, and mammary glands in animal experiments.

5. The side effect of crude extract of areca nut

Among the benefits of consuming areca nut, other studies have shown that these nuts are carcinogens so that they can cause oral malignancy lesions. Arecoline is the most common alkaloid found in areca nut which is known to cause cytotoxicity in mammalian cells *in vivo* and cause carcinogenicity. Some of the oral lesions that can be caused by crude areca nuts include:

5.1 Oral submucous fibrosis

Oral submucous fibrosis (OSF) is a chronic disease that produces hyperkeratosis, epithelial atrophy, tissue fibrosis, and precancerous lesions. Pathological characteristics include chronic inflammation, excessive collagen deposition in the connective tissues below the oral mucosal epithelium, local inflammation in the lamina propria

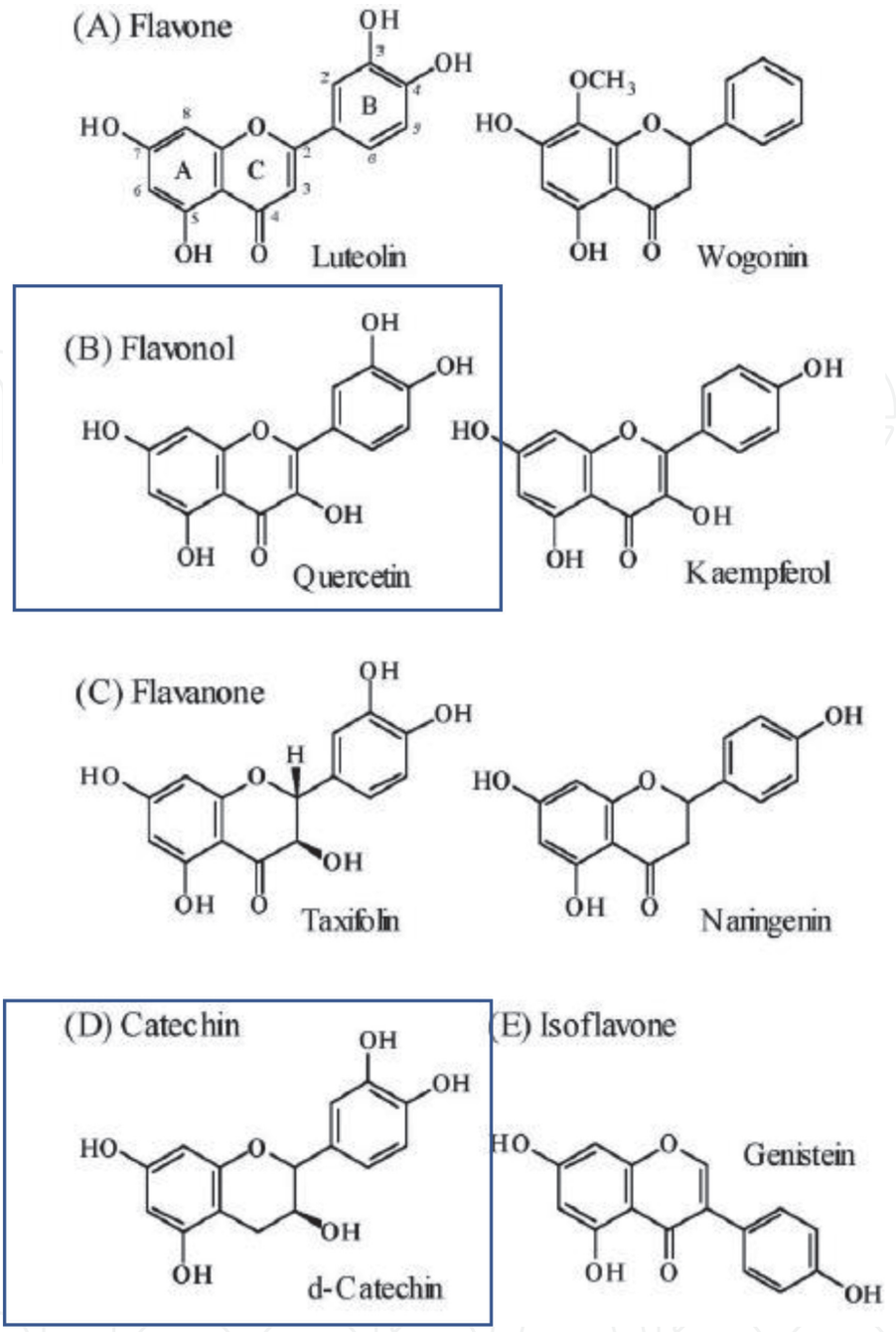


Figure 3.
Chemical structures of the flavonoid family. The areca nut contains flavonol (quercetin) and flavanol (catechin).

or deep connective tissues, and degenerative changes in the muscles. OSF patients experience a severe burning sensation in the mouth after ingesting spicy foods. Other symptoms of OSF include dry mouth, pain, taste disorders, restricted tongue mobility, trismus, dysphagia, and altered tone. This disease contributes significantly to mortality because of its high malignant transformation rate (1.5–15%). Previous research has shown that the arecoline in areca nuts can induce COX-2 expression in humans. It also suggests contributing to pathogenesis of OSF in betel chewers. Consistently, the elevated expression level of COX-2 has also observed in arecoline-treated HGF-1 cells and primarily cultured HGF cells in the study, suggesting these cells of different origins derived from oral cavity might have similar inflammatory responses upon exposure to arecoline, which in turn promote oral lesions and tumorigenesis.

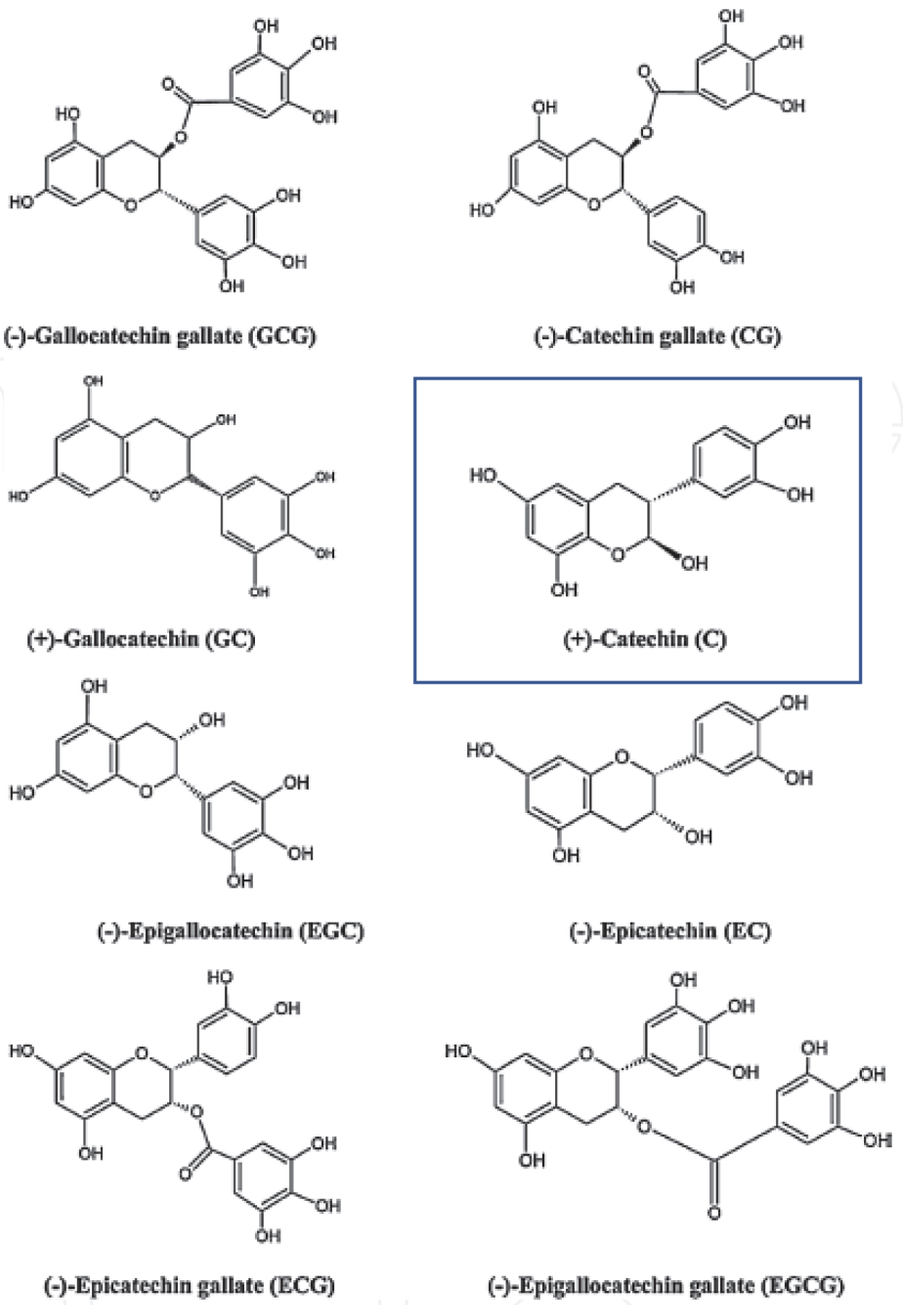


Figure 4.
Chemical structures of catechin. The areca nut contains (+)-Catechin.

5.2 Oral squamous cell carcinoma (OSCC)

Betel (areca nut) quid chewing is a widely prevalent habit correlated with a high incidence of oral cancer. A Previous study showed that the carcinogenic mechanism of action of betel quid chewing is caused by DNA damage induced by arecaidine and Cu (II). The Cu (II) is an important structural metal ion in chromatin; however, it is reported to be an important factor in DNA damage induced by many organic compounds. It was found that arecaidine alone had no significant effect on inducing DNA damage, but it caused significant DNA double strand breaks in the presence of Cu (II) ions under alkaline conditions. Further studies showed that reactive oxygen species were generated and Cu (II) was formed in the reaction. Areca nuts contain arecoline and arecaidine, both of which can cause mutation. When chewing areca nut, arecoline dissolves in saliva and its concentration can reach 140 g/mL. arecoline can cause cytotoxicity and genotoxicity for multicultured human cell lines and

inhibits oral mucosal and gingival fibroblast which can cause epithelial damage and delayed wound healing.

The areca nut does not contain carcinogenic substances, but this carcinogenic effect is caused by nitrosamines product in the long term and uncontrollable. Nitrosamines are produced by nitrosation of the alkaloids in dried stored nuts, when in the mouth, and especially in the acid conditions of the stomach, in the presence of nitric oxide generated by bacteria [39]. The combination with nitric oxide, produced by bacteria, causes the production of methylnitrosaminoproprionitrile which is proved to cause carcinogenesis in animal studies [39]. This endogen nitrosation is higher significantly in a patient with bad oral hygiene [40]. If areca nut is combined with tobacco, then chewed by people with bad oral hygiene, there will be a very high accumulation of nitrosamines product [41]. This process usually occurs continuously for the long term because the seeds have addictive properties. Some studies also reported increased reactive oxygen species such as hydroxyl oxide in the oral cavity caused by a combination of polyphenol auto-oxidation from areca nut with high alkaline pH of slaked lime (Calcium hydroxide paste) [42]. If the areca nut is chewed with *Piper betel* and slaked lime, these two materials will cause mucous membrane erosion, so that carcinogenic substance could easily penetrate cells through the mucous membrane [43]. Some part of the community in India and Pakistan use industrial packaging areca nut called *gutka* and *pan masala*. *Gutka* contains areca nut, *Piper betel*, tobacco, and slaked lime, while *pan masala* was prepared without tobacco. Approximately 40% of *gutka* and *pan masala* packaging are contaminated with aflatoxin which has carcinogenic properties from *Aspergillus flavus*, *Aspergillus niger,* and *Rhizopus spp.* fungi [44]. The occurrence of the OSCC's risk depends greatly on the composition of the compound which determines the quality of the seeds, the method of using seeds which are associated with oral hygiene, duration of use, the presence or absence of toxin caused by fungi contamination in the seeds, and the presence or absence of other carcinogenic substances such as tobacco and slaked lime. The occurrence of OSCC could also be influenced by several factors such as intrinsic factors (tumor suppressor gene abnormality or mutation and oncogene) and extrinsic factors (tobacco smoking, vitamin A and iron deficiency, candidiasis, viral infection, and immunosuppression).

The dried areca nut, gambir, and calcium hydroxide wrapped in betel leaf are usually used as symbol of respect for guests in traditional ceremonies in Aceh, Indonesia.

6. Role of catechin in cancer mechanisms

The term catechins are commonly used to refer to the family of flavonoids and the subgroup flavan-3-ols or simply, flavanol. Catechins are differentiated from the

ketone-containing flavonoids such as quercetin and rutin, which are called flavonols. High concentrations of catechin can be found in fresh tea leaves, red wine, broad beans, black grapes, apricots, and strawberries [45].

	Role of catechin in cancer mechanisms
Initiation Stage	• Catechins neutralize the procarcinogens by inhibiting the activity of cytochrome P450 enzyme and modulating free radicals [46]. • Catechins inhibit the activity of Nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome c reductase. • Epigallocatechin gallate (EGCG) could interact with hepatic cytochrome P450 and inhibit the P450-dependent mixed-function oxidase enzymes in the skin and liver [47]. • The epicatechin derivative structure is capable of inhibiting microsomal enzyme system derived from the catechin hydroxyl group. The pyrogallol structure causes catechin molecules to have a strong metal-chelating ability that can bind to metal transition ions and act as preventive antioxidants [48]. • The EGCG reduces cell proliferation and induces apoptosis in low-dose H_2O_2 (10 M)-treated colon carcinoma cells and downregulates 12-O-tetradecanoylphorbol-13-acetate-mediated oxidative stress in cervical carcinoma cells [49]. • The EGCG can suppress the growth of HepG2 human HCC cells. The oral administration of green tea for 1 year can inhibit the progression of high-grade prostate intraepithelial neoplasia to prostate cancer [50].
Promotion Stage	• The inhibitory mechanism of the promotion stage is divided into three processes, namely the intervention of intracellular signaling pathways, increases the caspase activity, and cell cycle modulation. • Catechins inhibit phosphorylation of extracellular signal-regulated protein kinases (ERK)-1 and 2 and suppress the activity of p38 MAPKs in human fibrosarcoma cells [51]. The ERK enzymes are important transducers of proliferation signals. • Catechin hydrate exhibits anticancer effects by blocking the proliferation of MCF7 cells and inducing apoptosis in part by modulating expression levels of caspase-3, −8, and − 9 and p53 [52]. • (−)-Epigallocatechin-3-gallate has potential as a novel therapeutic agent for patients with B-cell malignancies including multiple myeloma via induction of apoptosis mediated by modification of the redox system. • Catechins can also inhibit the cell cycle. The cell cycle is controlled by numerous mechanisms ensuring correct cell division [53]. The mechanisms are regulation of cyclin-dependent kinases (CDK) by cyclins, CDK inhibitors, and phosphorylating events [53]. • **Cell-cycle dysregulation** is a hallmark of tumor cells. The ability of normal cells to undergo cell-cycle arrest after damage to DNA is crucial for the maintenance of genomic integrity [54]. • The biochemical pathways that stop the cell cycle in response to cellular stressors are called **checkpoints.** • Defective checkpoint function results in genetic modifications that contribute to carcinogenesis. The regulation of checkpoint signaling also has important clinical implications because the abrogation of checkpoint function can alter the sensitivity of tumor cells to chemotherapeutics.
Progression Stage	• Metastasis requires down-regulation of cell adhesion receptors necessary for tissue-specific, cell–cell attachment, as well as up-regulation of receptors that enhance cell motility. • Inhibition of migration and invasion of tumor cells could be a target of anticancer therapy. • Catechins can inhibit MMP-2 and MMP-9 in endothelial cells [55]. MMP-2 and MMP-9 secretions are elevated in several types of human cancers and their elevated expression has been associated with poor prognosis. • **Angiogenesis**, the development of new capillaries from preexisting blood vessels, is required in physiological processes such as wound healing and pathological conditions including tumor growth and metastases.

Role of catechin in cancer mechanisms
• **Tumor angiogenesis** is a complex process that consists of several steps including the secretion of angiogenic factors by tumor and host cells, activation of proteolytic enzymes, endothelial cell migration, invasion, endothelial cell proliferation, and capillary formation. • Vascular endothelial growth factor (VEGF) and its receptors have been known as important angiogenic factors and are commonly overexpressed in several types of human cancers. • Catechins especially EGCG is proved to inhibit tumor growth and angiogenesis by the down-regulation of VEGF expression in serum-deprived HT29 human colon cancer cells [56]. • Catechin in green tea extract inhibits angiogenesis partly through the disruption of STAT3-mediated transcription of genes, including VEGF [57]. • Several members of the signal transducers and activators of transcription (STAT) family play a role in tumorigenesis. The STAT3 activity is commonly upregulated in breast cancer and regulates the expression of angiogenic genes including VEGF and MMP9 [57].

7. Antioxidant activity of areca nut

Antioxidants protect cells from deleterious effects of oxidation and are also employed as dietary supplements to neutralize the adverse effects of oxidative stress. Many of the natural antioxidants of interest are of plant origin and belong to bioactive compounds in the phenolic and polyphenolic class. The phytochemical contents of areca nut mostly come from phenolic and flavonoid content which produce antioxidant activity. Total phenolic content test using the Folin–Ciocalteu method was performed based on oxidation–reduction mechanism. Methanol is the best solvent for areca nut compared to petroleum ether, ethyl acetate, and water, and was used in this study [58]. The value of total phenolic concentration in 1 gram extract is 80.3 mg TAE/gr extract. The highest phenolic concentration was usually found in methanol, acetonide, and water solvent. This concentration is depended on how big the solvent's polarity is used at extraction. The high phenolic solubility in the polar solvent showed a high concentration of phenolic content in the extract. When compared with literature using the same method, the phenolic content of areca nut in the study is lower than that of a study conducted in Assam, India (146.7 mgTAE/g extracts) and Hainan province, Taiwan (167.70 mgTAE/g extracts) [5, 58]. Total flavonoid test using Dowd modification method also revealed a higher content of flavonoid (238.5 mg CEmg/gr extract). This number is higher than that reported by Zhang et al. and Wang et al., which was 77.36 and 10.45–142.65 mg CE mg/gr extract, respectively. This indicates that the variation of polyphenol content depends on the geographical locations where the plant grows. This variation is also affected by species and the characteristics of the nut used in the study, including freshness, maturity, and methods of drying.

The flavonoid in areca nut extract has antioxidant and also pro-oxidant activity. These two activities were also possessed by other herbal plants, *curcumin* [59, 60]. Lower doses of *curcumin* (12.5 μM) has the properties of reactive oxygen species scavenging, anti-inflammatory, apoptosis induction, and proliferation inhibition in myeloid leukemic cells, but in a higher dose and long term, the metabolite contents of *curcumin,* which is lipophilic or water-insoluble, could increase the level of cellular reactive oxygen species that causes carcinogenic potential through oxidative DNA damage or metal-mediated *DNA* damage at P450 cytochrome [61, 62]. This damage occurred because of the presence of Cu(II)-CYP2D6 which caused the damage of the DNA, especially 5-TG-3, 5-GC-3, and GG sequences [61]. *Curcumin* could induce lung cancer by increasing reactive oxygen species resulting in disarray

between mitogen-activated protein kinase, NF-jb, and p53, causing genetic mutation and oxidative stress [63]. This finding concluded that the antioxidant effect which was started by an oxidative stimulus, depending on time, dose, and certain cancer type, could also cause unwanted side effects [63].

Catechins are phytochemical compounds found in high concentrations in a variety of plant-based foods and beverages. Catechins are classified as flavanols. Catechin is the highest phenolic compound in the areca nut extract (2.79 mg/g). In comparison with catechin, quercetin is found in much smaller amounts in the areca nut extract (0.14 mg/g). The areca nut extract from Aceh, Indonesia, has a low level of tannin (0.007%) through the titrimetric analysis. This finding showed different results with less amount than areca nut from various districts in Karnataka, India, showed 1.13%–3.39% tannin in areca nut extract with the same technique [29]. Tannin is a polyphenol compound of plant origin, bitter in taste, which reacts with and coagulates protein, or various other compounds including amino acids and alkaloids.

The main characteristic of an antioxidant is its ability to trap free radicals. The antioxidant activity of an extract can be measured by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical method. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. The substance which is performs this reaction can be considered as antioxidants and therefore radical scavengers [64]. The DPPH test is a direct and reliable method for determining radical scavenging action. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. The result of the antioxidant activity curve showed linear line formula so the EC_{50} value was acquired. The EC_{50} value is the extract concentration which was able to catch 50% free radical. The EC_{50} value was measured from the association curve between the percentages of radical catcher DPPH against the concentration of the treatment's solution. This value is inversely proportional to antioxidant extract capability. The higher the antioxidant activity, the lower EC_{50} would be. The study showed that the EC_{50} value of areca nut extract was 15.95 μg/mL (**Figure 5**). The polyphenol could

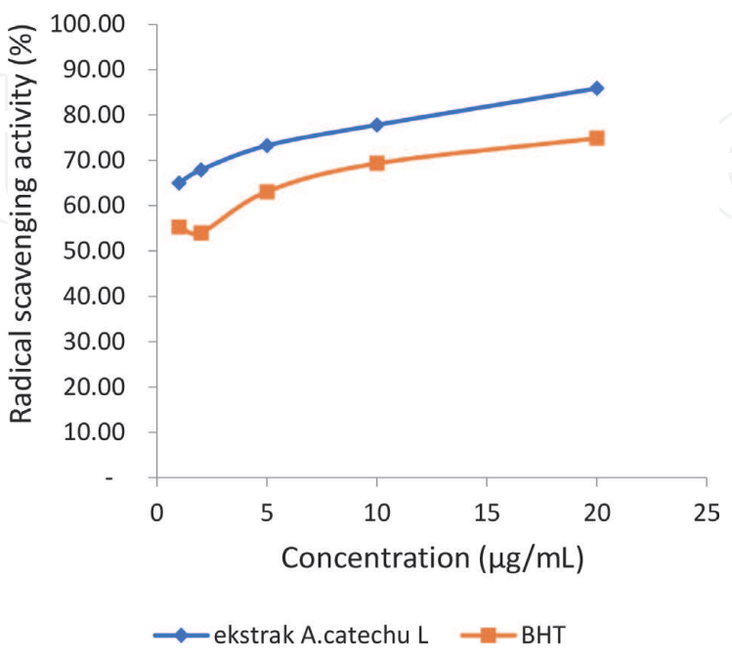


Figure 5.
Antioxidant activity estimated by DPPH method. The linear curve of areca nut extract has similar linearity with BHT control. The dosage used was 1–20 μg/mL.

dispose of free radicals by becoming a hydrogen donor so the free radical chain reaction was broken. The EC_{50} value of the extract was smaller than Butylated Hydroxytoluene (BHT); synthetic antioxidant posing as control. The result of the study showed stronger antioxidant activity of areca nut compared to BHT control, so it could be concluded that the activity potency of the combination of several phenolic compounds in the extract could work synergic and resulted in more potent antioxidant than the activity of one isolate in the extract [38]. Polyphenolics had a stimulation effect on mitochondria's activity, so it could be more efficient in creating energy and preventing free radicals. It can increase the cell viability graph over 100% in human keratinocyte (HaCat) cell line [28].

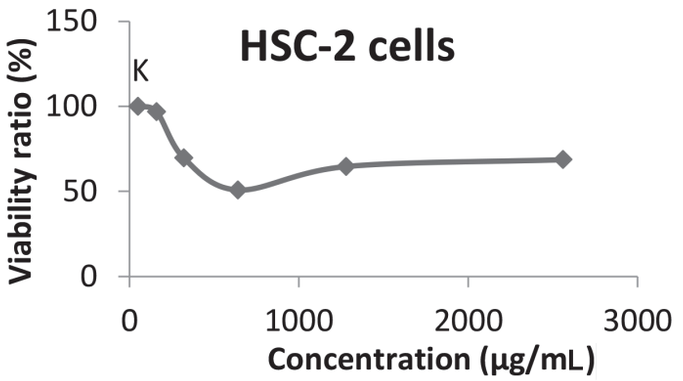
8. The cytotoxicity and immunomodulatory activity of areca nut to the human health

8.1 Cytotoxicity activity of areca nut

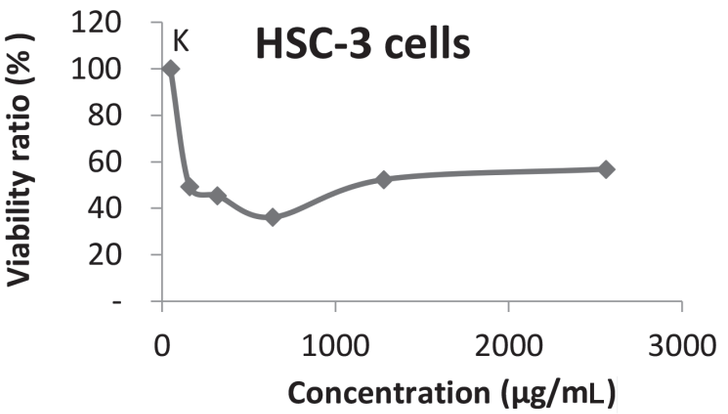
The majority of the community prefer to choose herbal medicine because the natural ingredient is considered to be safer and cheaper than chemical drugs. The precipitating factors of the increasing use of herbal medicine in developed countries are the side effects of chemical drugs, the high cost of modern medicine, and the increasing life expectancy when the prevalence of chronic disease had increased, so herbal medicine becomes an alternative treatment that is believed to cover all classes of the community especially in Indonesia [65]. In Taiwan and South-Eastern Asia, areca nut chewing has been associated with the development of oral squamous cell carcinoma (OSCC) through epidemiological studies and has been classified as a human carcinogen by the IARC (2004) [6].

MTS assay was done to observe the cytotoxicity effect of areca nut extract in HSC-2, HSC-3, and HaCat cells. Areca nut has a stimulation effect on mitochondria's activity, so it could be more efficient in creating energy and preventing free radicals. It can increase the cell viability graph over 100% in HaCat cell line by MTS assay. The areca nut extract in a certain dose could increase respiration and metabolism in the HaCat cell line [28]. Cytotoxicity of the extract was displayed with the viability percentage. The result showed that IC_{50} of areca nut extract in HSC-2 cells was 629.50 μg/mL and the IC_{50} value in HSC-3 occurred in lower concentration which was 164.06 μg/mL. The cytotoxicity effect started from the smallest concentration which was between 160–640 μg/mL, but at a concentration higher than 1280 μg/mL, the extract started to show proliferative activity. Areca nut extract provided a stronger cytotoxicity effect in HSC-3 cells than in HSC-2 cells. The cytotoxicity graph of the three cell lines is shown in **Figure 6.**

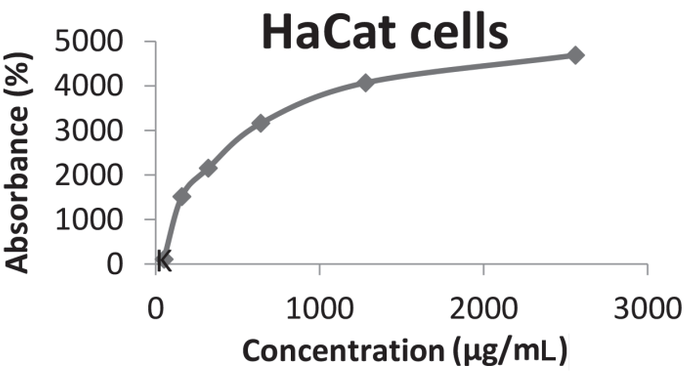
MTS assay showed increased cellular viability in doses higher than IC_{50}, which was 16.2% followed by 4.5% in HSC-3 cells and an increase of 13.8% and 4% in HSC-2 cells. This was caused by strong antioxidant activity in the extract. Polyphenol had a direct stimulation effect on mitochondria's activity, so it was more efficient in producing energy and free radical scavenging. This was probably caused by the high extracellular formazan intensity from tetrazolium reduction by dehydrogenase succinate enzyme in cells performing respiration and metabolism. The more energy and cellular respiration product, the more formazan would be formed. Areca nut extract did not cause cytotoxicity in HaCat cell lines. This study showed that areca nut could induce formazan in large numbers so it showed a large increase in cell number. This was not caused by the high HaCat cells viability, but because of the high absorbance value by following the dense formazan color which was influenced by cellular respiration and metabolism. This absorbance value was read



a.



b.



c.

Figure 6.
The result of MTS assay in HSC-2, HSC-3, and HaCat cell lines. Areca nut extract has a stronger cytotoxicity effect in HSC-3 cells (b) than in HSC-2 cells (a). The extract does not show a cytotoxicity effect in HaCat cells (c).

based on 490 nm wavelength. The denser the color of formazan's product, the higher the absorbance value would be. Although this study did not perform the test for Nicotinamide Adenosine Dinucleotide Hydrogen (NADH) content in the extract, it is possible that areca nut extract might contain NADH which could provide additional energy for the cells. NADH is an active coenzyme form of vitamin B3 (Niacin) which has an important role in the nervous system. This

vitamin is found in all living cells of plants, animals, and humans. Tharakan et al. showed that *Tricopus zeylanicus* containing NADH had antioxidant activity by inducing lipid peroxidase and inhibiting lipoxygenase activity [66]. Further studies are needed to detect NADH content in areca nut.

The flavonoid in areca nut extract has antioxidant and also pro-oxidant activity. These two activities were also possessed by other herbal plants, *curcumin* [59, 60]. Lower doses of *curcumin* (12.5 μM) has the properties of reactive oxygen species scavenging, anti-inflammatory, apoptosis induction, and proliferation inhibition in myeloid leukemic cells, but in a higher dose and long term, the metabolite contents of *curcumin,* which is lipophilic or water-insoluble, could increase the level of cellular reactive oxygen species that causes carcinogenic potential through oxidative DNA damage or metal-mediated DNA damage at P450 cytochrome [61, 62]. This damage occurred because of the presence of Cu(II)-CYP2D6 which caused the damage of the DNA, especially 5-TG-3, 5-GC-3, and GG sequences [61]. *Curcumin* could induce lung cancer by increasing reactive oxygen species resulting in disarray between mitogen-activated protein kinase, NF-jb, and p53, causing genetic mutation and oxidative stress [63]. This finding concluded that the antioxidant effect which was started by an oxidative stimulus, depending on time, dose, and certain cancer type, could also cause unwanted side effects [63].

The principle of the MTS assay method was determined by a reduction–oxidation reaction occurring in cells. MTT/MTS reagent was reduced into formazan salts by succinate dehydrogenase enzyme found in living mitochondria cells. This reaction was allowed to take place for 4 hours then stopper reagent was added in MTT assay. The stopper reagent will lysate the cell's membrane so that formazan salts could get outside of the cell, and it could be dissolved. The MTS assay does not need a stopper reagent because formazan could dissolve in the culture medium with the addition of PMS. The formazan salt, that was in extracellular, was quantified with a spectrophotometer and measured in form of absorbance (**Figure 7**).

The higher the absorbance, the higher cell viability would be. The IC_{50} value between HSC-2 and HSC-3 cells had quite a large difference range. This difference showed the selective toxicity difference and type of cell death in some OSCC cell lines during exposure by natural anticancer or synthetic agent. The flavonoid compound showed weak cytotoxicity activity against HSC-2 cells so the IC_{50} value was higher [67, 68]. The factors of the substance in plants that influenced the cytotoxicity capability were the presence or absence of hydrophilic and hydrophobic groups in one same molecule, the presence or absence of isoprenyl groups, the presence or absence of polycyclic and/or halogen structure, the most condensed structure (low molecular weight is more cytotoxic) and lipophilicity. The factors from inside the cells which could probably cause this cytotoxicity was the difference

Figure 7.
The cells preparation in MTS assay.

of protein expression which was resistant against some anticancer agent and expression of the drug's metabolism enzyme or natural substances [68]. Environmental factors were serum type, the presence of metal ion, oxygen concentration, and external pressure [68]. The success of the MTS assay depended greatly on a cell's metabolism and respiration capability. This study also showed the morphology of HSC-2 cells which was different from HSC-3 cells. The HSC-2 cells had bigger, wider cytoplasm and tighter cell junction than HSC-3 cells that probably could cause weaker extract's cytotoxicity activity than in HSC-2 cells so the IC_{50} value of HSC-2 was higher than HSC-3 cells.

Analysis of apoptotic cells using flow cytometry demonstrated that areca nut can induce apoptosis in oral cancer cell lines, HSC-2 and HSC-3 cell lines [14]. The caspase-3 activity as an effector caspase is shown to be related to late apoptosis activity because of the increase of caspase-3 with increasing late apoptotic cells percentage in both cells after areca nut treatment. Analysis of caspase activity confirmed that apoptosis might be the major mechanism of cell death induced by areca nut. As far as we know, there is no similar report regarding the caspase-3 activity induced by areca nut extract, but this result is similar to several previous studies that used plants containing flavonoids to increase caspase activity in cancer cells [41–43]. This finding may have biological implications in cancer treatment. The caspases inside cells are in an inactive form (procaspase), but activation induces the production of other caspases leading to cell death through proteolytic activity [44, 45]. Caspase-3 activation is a crucial component in the apoptotic signaling cascade. The apoptosis pathway involved in areca nut-induced cell death in both cancer cell lines may be through the extrinsic and intrinsic pathways. The areca nut also caused the cell-cycle arrest in HSC-3 and HSC-2 cell lines. The areca nut inhibited cell proliferation by the enhancement of Ki-67 after 24 hours of extract treatment in both cells [69].

8.2 Immunomodulatory activity

The areca nut extract was found to increase the white blood cell count post-challenge with *Staphylococcus aureus* induction significantly indicating that the extract could stimulate the hemopoietic system. Another study found that areca nut extract can induce calcium signals in at least three immune cell lines and human primary immune cells (PBMCs), inducing the production of pro-inflammatory cytokines. Further separation of the PBMCs into T cells, B cells, and monocytes would potentially be of interest in elucidating specific responses to each cell subtype [70]. However, areca nut extract can also induce antigen-specific immune responses and promote inflammatory reactions *in vivo*, which may contribute to immune deregulation associated with areca-related diseases [71]. In fact, the study of areca nut as an immunomodulatory drug still causes various results. It depends on the content of polyphenolic compound in the areca nut extract which greatly affects the efficacy of the nut.

The areca nut can also increase the activity and capacity of macrophages. The process by which a cell ingests and disposes of foreign material, including microorganisms is called phagocytosis. In normal conditions, most phagocytes are circulating in the blood and when there is an inflammation, the phagocytes will leave the bloodstream and migrate to the site of inflammation. The circulation in the capillaries and venules is rapidly moving with the red blood cells in the mainstream and neutrophils and other leukocytes tending to flow more slowly along the vessel's periphery. Monocytes and macrophages have the same functions as neutrophils but for a longer time and in a later stage of the inflammatory response. Monocytes are produced in the bone marrow, enter the circulation, and migrate to the

inflammatory site, where they develop into macrophages. Macrophages are more active as phagocytes than their monocytic precursors. Macrophages, particularly those residing in the tissue, are often important cellular initiators of the inflammatory response.

Several bacteria are resistant to killing by granulocytes and can even survive inside macrophages. Microorganisms such as *Mycobacterium tuberculosis*, *Salmonella typhi,* and *Mycobacterium leprae* can remain dormant or even multiply inside the phagolysosomes of macrophages. However, the bactericidal activity of macrophages can be markedly increased with the help of inflammatory cytokines produced by cells of the acquired immune system (a subset of T lymphocyte) or cells activated through Toll-like receptors. Macrophage activation results in increased phagocytic activity, the size of itself, plasma membrane area, glucose metabolism, and a number of lysosomes. The activation of leukocytes, monocytes, and macrophages which is induced by *S. aureus* and treated with areca nut is shown in **Figure 8**.

The areca nut extract probably stimulates the proliferation of macrophages, which in turn leads to the activation of macrophage activity. Further study is needed to find out how extract can increase macrophage activity and capacity.

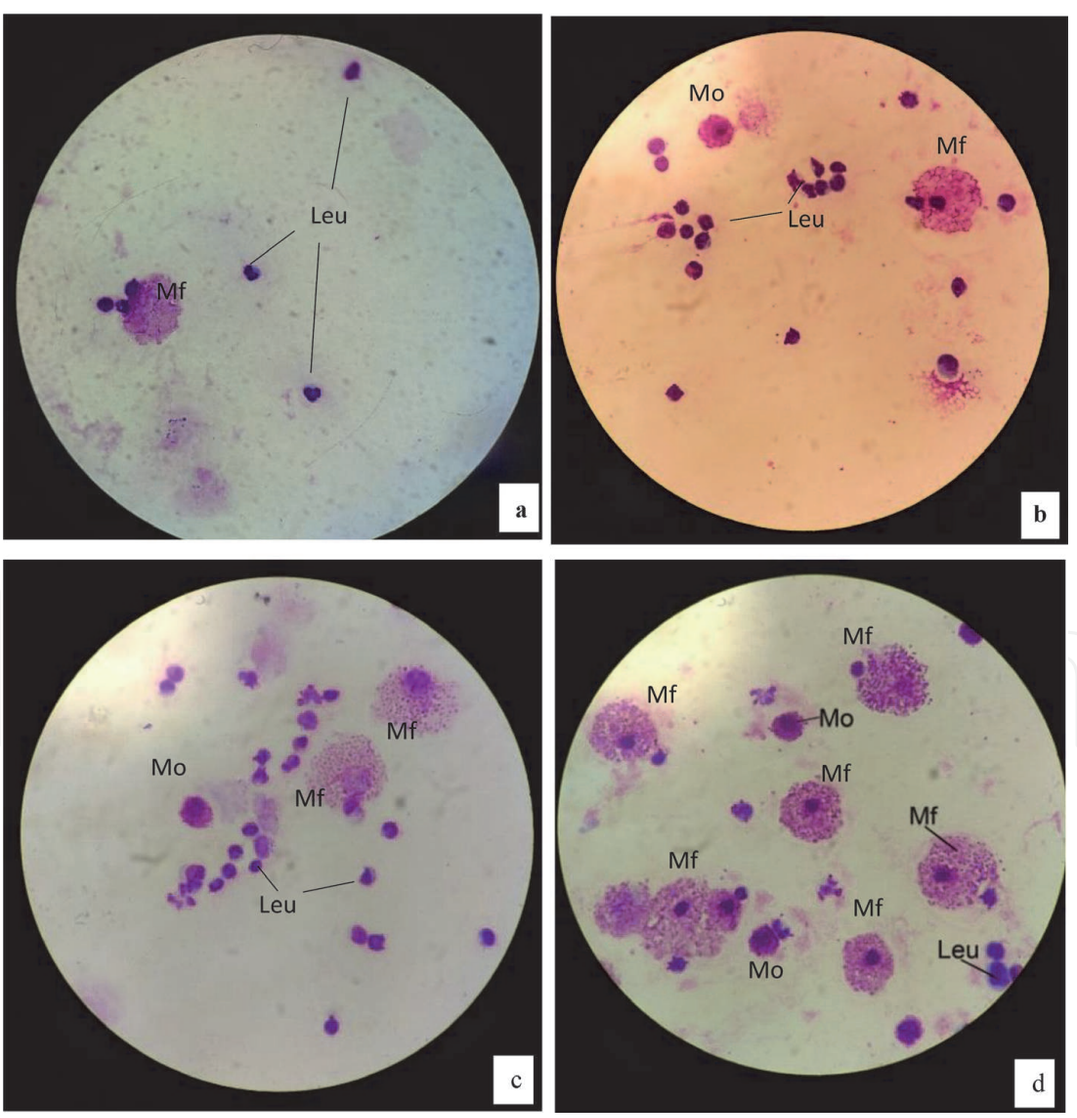


Figure 8.
Intraperitoneal fluid in Giemsa staining. a. The control group showing leukocytes and monocytes, b. Group 2 (500 mg/kg BW) showing an increase in the number of leukocytes, and monocytes, c. Group 3 (1000 mg/kg BW) showing leukocytes, monocytes, and macrophages, d. Group 4 showing macrophages. Mf = macrophage Fagocyte, Mo = monocyte, Leu = leukocyte.

However, not only is the effect of the treatment given, but the increase in macrophage activity against *S. aureus* infection might also be caused by the internal factor of the macrophage itself. The previous study reported the role of macrophage transmembrane expression that suppressed the *S. aureus*-induced production of nitric oxide and proinflammatory cytokines in mouse macrophages [72]. Moreover, it has been reported by several groups that this bacterium can invade and survive within a variety of cells such as neutrophils, macrophages, T-lymphocytes, epithelial cells, endothelial cells, fibroblasts, and osteoblasts which may be related to the intracellular persistence of bacteria within host cells [73–75]. The markers of biochemical examination (ureum, creatinine, SGOT, and SGPT) did not show changes in liver and kidney in mice after two weeks of treatment and one hour before and post-challenge with *S. aureus*. This study is in line with previous studies which revealed that the areca nut consumed in the long term in humans does not cause hepatotoxicity [76, 77]. However, another study showed that raw areca nut given for 28 days caused mild hepatotoxicity and nephrotoxicity in mice [78].

9. Conclusions

This chapter provides an overview of the characteristics of the areca nut and its impact on human health, especially in an anticancer and immunomodulatory effect. Areca nuts from Aceh, Indonesia, contain flavonoids, phenolics, catechin, quercetin, and a small percentage of tannins which contribute to antioxidant activity. The areca nut has anticancer potential activity so it can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy-induced toxicity. Areca nuts also show immunomodulatory activity which can increase the body's immune system.

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References

[1] Hamsar MN, Ismail S, Mordi M, Ramanathan S, et al. Antioxidant capacity and the effect of different parts of areca catechu extracts on gluthatione-S-Transferase activity invitro. Free Rad Antiox 2011;1(1):28–33.

[2] Jaiswal P, Kumar P, Singh VK, Singh DK. *Areca catechu* L.: A valuable herbal medicine againts different health problems. Res J Med Plant 2011;5(2): 145–152.

[3] Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agri Food Chem 1998;46(9):4113–4117.

[4] Ahsan H, Ali A, Ali R. Oxygen free radicals and systemic autoimmunity. Clin Exp Immunol 2003;31:398–404.

[5] Zhang WM, Wei J, Chen WX, Zhang HD. The chemical composition and phenolic antioxidants of *areca* (*areca catechu* L.) seeds. ICABE 2011;1-2:16–22.

[6] Huang PL, Chi CW, Liu TY. Effects of Areca catechu L. containing procyanidins on cyclooxygenase-2 expression in vitro and in vivo. Food Chem Toxicol 2010;48(1):306–313.

[7] Xing Z, Jiao W, Zhuang H, Li MW, Fu DH. Antioxidant and cytotoxic phenolic compounds of areca nut (*Areca catechu L.*). Chem Res Chinese Universities 2010;26(1):161–164.

[8] Staples GW, Bevacqua RF. Spesies profiles for pacific island agroforestry. Areca catechu (betel nut palm): www.traditionaltree.org; 2006. p. 1–24.

[9] IARC. WHO-biennial report. International agency for research on cancer, part I. 2004:1–192.

[10] Kim BJ, Kim JH, Kim HP, Heo MY. Biological screening of 100 plant extracts for cosmetic use (II): Anti-oxidative activity and free radical scavenging activity. Int J Cosmetic Sci 1997;19:299–307.

[11] Kook K, Lee JJ, Cho J, Park, Choi JD. The effects of *Areca Catechu* L Extract on Anti-Inflammation and AntiMelanogenesis. Int J Cosmetic Sci 1999;21(4):275–280.

[12] Lee KK, Choi JD. The effects of *Areca catechu* L extract on antiaging. Int J Cosmetic Sci 1999;21(4):285–295.

[13] Bhandare AM, Kshirsagar AD, Vyawahare NS, Hadambar AA, Thorve VS. Potential analgesic, anti-inflammatory and antioxidant activities of hydroalcoholic extract of Areca catechu L. nut. Food Chem. Toxicol 2010;48:3412–3417.

[14] Sari LM, Subita GP, Auerkari EI. Areca nut extract demonstrated apoptosis-inducing mechanism by increased caspase-3 activities on oral squamous cell carcinoma F1000 Res 2019;7(723):1–10.

[15] Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, et al. A metabolic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chem Res Toxicol 2006;19:818–827.

[16] Zhou J, Sun Q, Yang Z, Zhang J. The hepatotoxicity and testicular toxicity induced by arecoline in mice and protective effects of vitamins C and E. Korean J Physiol Pharmacol 2014;18(2): 143–148.

[17] Chuerduangphui J, Ekalaksananan T, Chaiyarit P, Patarapadungkit N, Chotiyano A, Kongyingyoes B, et al. Effects of arecoline on proliferation of oral squamous cell carcinoma cells by

dysregulating c-Myc and miR-22, directly targeting oncostatin M. PLoS ONE 2018;13(1):e0192009.

[18] Liu R. Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. J. Nutr 2004;134 (12):3479S–3485S.

[19] Min KJ, and Kwon TK. Anticancer effects and molecular mechanisms of epigallocatechin-3-gallate. IMR 2014;3: 16–24.

[20] Lambert JD, and Elias RJ. The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. Arch Biochem Biophys 2010;501:65–72.

[21] Roychoudhury S, Agarwal A, Virk G, and Cho CL. Potential role of green tea catechins in the management of oxidative stress-associated infertility. Reprod Biomed online 2017;34(5):487–498.

[22] Ren W, Qiao Z, Wang H, Zhu L, and Zhang L. Flavonoids: Promising Anticancer Agents. Med Res Rev 2003; 23(4):519–534.

[23] Shimizu M, Shirakami Y, Sakai H, Kubota M, Kochi T, et al. Chemopreventive Potential of Green Tea Catechins in Hepatocellular Carcinoma. Int J Mol Sci 2015;16(3): 6124–6139.

[24] Iwasaki R, Ito K, Ishida T, Hamanoue M, Adachi S, et al. Catechin, green tea component, causes caspase independent necrosis-like cell death in chronic myelogenous leukemia. Cancer Sci 2009;100(2):349–356.

[25] Khiewkamrop P, Phunsomboon P, Richert L, Pekthong D, and Srisawang P. Epistructured catechins, EGCG and EC facilitate apoptosis induction through targeting de novo lipogenesis pathway in HepG2 cells. Cancer Cell Int 2018;18 (46):1–13.

[26] Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK, Viswanathan S, Kameswaran L. Anti-inflammatory and C.N.S. depressant activities of xanthones from Calophyllum inophyllum and Mesua ferrea. Ind J Pharmac 1980;12(3):181–191.

[27] Chilpa RR, Baggio CH, Solano DA, Muniz EE, Kaufmann FC, et al. Inhibition of gastric H+, K+,-ATPase activity by flavonoids coumarins and xanthones isolated from Mexican medicinal. J Ethnopharm 2006;105:167–172.

[28] Sari LM, Subita GP, Auerkari EI. Potential antioxidant and cytotoxic activity of areca nut (*Areca catechu* L.) extract in human oral squamous cell carcinoma and keratinocyte cells. Asian J Pharm Clin Res 2017;10(10):286–291.

[29] Gurumurthy B. Diversity in Tannin and Fiber Content in Areca Nut (*Areca catechu*) Samples of Karnataka, India. Int J Curr Microbiol App Sci 2018;7(1): 2899–2906.

[30] Wolfe KL, Liu RH. Structure-activity relationships of flavonoids in the cellular antioxidant activity assay J Agric Food Chem 2008;56:8404–8411.

[31] Sazwi NN, Nalina T, Rahim AZH. Antioxidant and cytoprotective activities of *Piper betle, Areca catechu, Uncaria gambir* and betel quid with and witouth calcium hydoxide. BMC Complem Altern M 2013;13(351):1–12.

[32] Wetwitayaklung P, Paechamud T, Limmatvapirat C, et al. The study of antioxidant capacity in various parts of *Areca catechu* L. Naresuan Univ J 2006; 14(1):1–14.

[33] Khiewkamrop P, Phunsomboon P, Richert L, Pekthong D, Srisawang P. Epistructured catechins, EGCG, and EC facilitate apoptosis induction through targeting de novo lipogenesis pathway

in HepG2 cells. Cancer Cell Int 2018;18 (46):1–13.

[34] Zaveri NT. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci 2006;78:2073–2080.

[35] Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives, and clinical applications. Biochem Pharmacol 2011;82(12):1807–1821.

[36] Mayr C, Wagner A, Neureiter D, Pichler M, Jakab M, et al. The green tea catechin epigallocatechin gallate induces cell cycle arrest and shows potential synergism with cisplatin in biliary tract cancer cells. BMC Complement Altern Med 2015;15(194):1–7.

[37] Gerates L, Moonen HJJ, Brauers K, Wouters EFM, Bast A, et al. Dietary flavones and flavonols are inhibitor of poly (ADP-ribose) polymerase-1 in pulmonary epithelial cells J Nutr 2007; 137: 2190–2195.

[38] Ramamoorthy P, Bono A. Antioxidant activity, total phenolic, and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction processes. IJEST 2007;2(1):70–80.

[39] Bhisey RA, Boucher BJ, Hsi-Chen TH, Gajalakshmi V, Gupta PC, et al. Monographs on the Evaluation of Carcinogenic Risks to Humans.Betel-Quid and Areca-Nut Chewing and some Areca-Nut Related Nitrosamines. IARCS Monographs 2004;85:1–293.

[40] Nagao T, Ikeda N, Warnakulasuriya S, Fukano H, Yuasa H, et al. Serum antioxidant micronutrients and the risk of oral leukoplakia among Japanese. Oral Oncol 2000;36: 466–470.

[41] Carossa S, Pera P, Doglio P, Lombardo S, Colagrande P, et al. Oral nitric oxide during plaque deposition. Eur J Clin Invest 2001;31:876–879.

[42] Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. Mutagenesis 2004;19:251–262.

[43] Johnson NW, Jayasekara P, dan Amarasinghe HK. Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. Periodontology 2000. Singapura: John Wiley and Sons; 2011.

[44] Anttila A, Bhat RV, Bhond JA, Bhorgoff SJ, Bosch FX, et al. Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARCS Monographs 2002;82:171–174.

[45] Gadkari PV, Balaramana M Catechins: Sources, extraction and encapsulation: A review Food Bioprod Process 2014;xxx:1–17.

[46] Chen L, Zhang HY Cancer Preventive Mechanisms of the Green Tea Polyphenol (−)-Epigallocatechin-3-gallate. Molecules 2007;12:946–957.

[47] Mukhtar H, Wang ZY, Katiqan SK, Agarwal R. Tea components: antimutagenic and antigagenic effects. Prev Med 1992;21:351–360.

[48] Misaka S, Kawabe K, Onoue S, Werba JP, Giroli M, et al. Effects of green tea catechins on cytochrome p450 2B6, 2C8, 2C19, 2D6, and 3A activities in human liver and intestinal microsomes. Drug Metab Pharmacokinet 2013;28(3):244–249.

[49] Park IJ, Lee YK, Hwang JT, Kwon DY, Ha J, Park OJ. Green tea catechin controls apoptosis in colon cancer cells by attenuation of H_2O_2-stimulated COX-2 expression via the AMPK signaling

pathway at low-dose H_2O_2. Ann N Y Acad Sci 2009;1171:538–544.

[50] Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, et al. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: A preliminary report from a one-year proof-of-principle study. Cancer Res 2006;66:1234–1240.

[51] Maeda-Yamamoto M, Suzuki N, Sawai Y, Miyase T, Sano M, et al. Association of suppression of extracellular signal-regulated kinase phosphorylation by epigallocatechin gallate with the reduction of matrix metalloproteinase activities in human fibrosarcoma HT1080 cells. J Agric Food Chem 2003;51:1858–1863.

[52] Alshatwi AA. Catechin hydrate suppresses MCF-7 proliferation through TP53/Caspase-mediated apoptosis. J Exp Clin Canc Res 2010;29(167):1–9.

[53] Vermeulen K, Berneman ZN, Van Bockstaele DR. Cell Cycle and Apoptosis. Cell Prolif 2003;36(3):165–175.

[54] Stewart ZA, Westfall MD, Pietenpol JA. Cell-cycle dysregulation and anticancer therapy. Trends Pharmacol Sci 2003;2(3):139–145.

[55] Fassina G, Vene R, Morini M Mechanisms of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. Clin Cancer Res 2004;10:4865–4873.

[56] Jung YD, Kim MS, Shin BA, Chay KO, Ahn BW, Liu W, et al. EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br J Cancer 2001;84:844–850.

[57] Leong H, Mathur PS, Greene GL. Green tea catechins inhibit angiogenesis through suppression of STAT3 activation. Breast Cancer Res Treat 2009;117(3):505–515.

[58] Hannan A, Karan S, Chatterjee TP. A comparative study of invitro antioxidant capacity of different extract of *areca* seed collected from *Areca catechu* L plant grown in Assam. Int J Pharm Pharm 2012;4(2):420–427.

[59] Kong Y, Ma W, Liu X, Zu Y, Fu Y, et al. Cytotoxic Activity of Curcumin towards CCRF-CEM Leukemia Cells and Its Effect on DNA Damage. Molecules 2009;14:5328–5338.

[60] Gibellini L, Pinti M, Nasi M, Biasi SD, Roat E, et al. Interfering with ROS Metabolism in Cancer Cells: The Potential Role of Quercetin. Cancers 2010;2:1288–1311.

[61] Sakano K, Kawanishi S. Metal-mediated DNA damage induced by curcumin in the presence of human cytochrome p450 isozymes Arch Biochem Biophys 2002;405:223–230.

[62] Gupta SC, Hevia D, Patchva S, Park B, Koh W, et al. Upsides and downsides of reactive oxygen species for cancer: The roles of reactive oxygen species in tumorigenesis, prevention, and therapy. Antioxid Redox Signal 2012;16(11):1295–1322.

[63] Dance-Barnes ST, Kock ND, Moore JE, Lin EY, Mosley J, et al. Lung tumor promotion by *curcumin*. Carcinogenesis 2009;30(6):1016–1023.

[64] Roy S, Choudhury MD, Paul SB. Antioxidant potential of rhiome of alocasia decipiens schott. Asian J Pharm Clin Res 2013;6(2):120–122.

[65] Dewoto HR. Pengembangan Obat Tradisional Indonesia Menjadi Fitofarmaka. Maj Kedokt Indon 2007;57 (7):205–211.

[66] Tharakan B, Dhanasekaran M, Manyam BV. Antioxidant and DNA

Protecting Properties of Anti-fatigue Herb Trichopus zeylanicus. Phytother Res 2005;19:669–673.

[67] Okamura M, Shimada J, Sakagami H. Comparative Analysis of Cell Death Induction by Cisplatin and 5-FU in Human Oral Squamous and Hepatocellular Carcinoma Cell Lines. Anticancer Res 2008;28:253–260.

[68] Sakagami H, Kobayashi H, Chien CH, Kanegae H, Kawase M. Selective Toxicity and Type of Cell Death Induced by Various Natural and Synthetic Compounds in Oral Squamous Cell Carcinoma. In vivo 2007;21:311–320.

[69] LM Sari, Wulandari D, Bustami A, Subita GP, Auerkari EI. Tannin Screening, Phenolic Compounds Analysis, and Antiproliferative Activity of Areca Nut Extract by Decreasing Ki-67 Protein in Oral Squamous Carcinoma Cell Lines. Trop J Nat Prod Res 2020;4 (9):563–570.

[70] Faouzi M, Neupane RP, Yang J, Williams P, Penner R. Areca nut extracts mobilize calcium and release pro-infammatory cytokines from various immune cells. Sci Rep 2018; 1075:1–13.

[71] Wang CC, Lin HL, Wey SP, Jan TR. Areca nut extract modulates antigen-specific immunity and augments inflammation in ovalbumin-sensitized mice. Immunopharmacol Immunotoxicol 2011;33(2):15–22.

[72] Zhu B, Yu Y, Liu X, Han Q, Kang Y, et al. CD200 Modulates *S. aureus*-Induced Innate Immune Responses Through Suppressing p38 Signaling. Int J Mol Sci 2019;20(659):1–15.

[73] Hamza T, Li B. Differential responses of osteoblasts and macrophages upon *Staphylococcus aureus* infection. BMC Microbiol 2014;14(207): 1–11.

[74] Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, et al. A potential new pathway for *Staphylococcus aureus* dissemination: the silent survival of *S. aureus* phagocytosed by human monocyte-derived macrophages. PLoS One 2008;3(1):e1409.

[75] Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindberg FP. Survival of *Staphylococcus aureus* inside neutrophils contributes to infection J Immunol 2000;164(7):3713–3722.

[76] Singroha K, Kamath VV. Liver function tests as a measure of hepatotoxicity in areca nut chewers. J Dent Res Rev 2016;3(2):1–5.

[77] Pai SR, Shirke AJ, Gothoskar SV. Longterm feeding study in C17 mice administered saccharin coated betel nut and 1,4dinitrosopiperazine in combination. Carcinogenesis 1981;2: 175–177.

[78] Lohith TS, Shridhar NB, Dilip SM, Jayashree P, Suhasini K. Repeated dose 28-day oral toxicity study of raw areca nut extract in rats. Int Res J Pharm 2013; 4(5):238–240.